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SUBMERGED AQUATIC VEGETATION IN UPPER CHESAPEAKE BAY: STUDIES RELATED TO POSSIBLE CAUSES OF THE RECENT DECLINE IN ABUNDANCE[a]

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16. ABSTRACT

This paper provides a synthesis of research conducted on possible causes of the decline in abundance of submerged aquatic vegetation (SAV) in upper Chesapeake Bay beginning in the late 1960's. Three factors potentially were emphasized in this study: runoff of agricultural herbicides; erosional inputs of fine-grain sediments; nutrient enrichment and associated algal growth. Widespread use of herbicides in the estuarine watershed occurred contemporaneous with the SAV loss; however, extensive sampling of estuarine water and sediments during 1980-81 revealed that typical bay concentrations of herbicides (primarily atrazine) rarely exceeded 2ppb. However, normal concentrations (< 5 ppb) were shown experimently to have little measurable effect on plants. Historical increases in turbidity have been documented for some bay tributaries since the 1940's. Light (PAR) attenuation by suspended fine-grain sediments contributed more to total turbidity in bay shallows (< 1.5m) than did phytoplankton chlorophyll a. Evidence indicated that plant photosynthesis was light-limited for much of the day. Effects of the continual increase in nutrient enrichment of the bay (documented since 1930) were tested by experimentally fertilizing pond mesocosms at levels common to the upper estuary. Moderate to high nutrient loadings resulted in significant increases in growth of epiphytic and planktonic algae and decreases in SAV production.

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CONTENTS

Page

SUMMARY AND CONCLUSIONS vi

CHAPTER I. Concentrations of the Herbicides Atrazine and Linuron in Agricultural Drainage, Tributary Estuaries and Littoral Zones of Upper Chesapeake Bay

Acknowledgements .... I-ii
Introduction I-1
Methods I-2
Results and Discussion I-6
References I-15
Figures I-18
Tables I-29
Appendices I-34

CHAPTER II. Temporal Responses of a Submerged Vascular Plant and its Associated Autotrophic Community to Atrazine Stress in Estuarine Microcosms

Acknowledgements .... II-ii
Abstract II-1
Introduction II-2
Materials and Methods II-3
Results and Discussion II-6
References II-22

CHAPTER III. Effects of Atrazine and Linuron on Photosynthesis and Growth of *Potamogeton perfoliatus* and *Myriophyllum spicatum* in Estuarine Microcosms

Introduction III-1
Materials and Methods III-2
Results and Discussion III-9
Implications: Herbicides and Estuarine Vascular Plants III-29
References III-37
Appendices III-41

CHAPTER IV. Atrazine Uptake, Phytotoxicity, Release, and Short-Term Recovry for the Submerged Aquatic Plant, *Potamogeton perfoliatus*

Abstract IV-1
Introduction IV-2
Materials and Methods IV-3
Results and Discussion IV-5

Page

Conclusions.. IV-15
References... IV-16

CHAPTER V. Uptake and Phytotoxicity of Soil-Sorbed Atrazine for the Submerged Aquatic Plant, Potamogeton perfoliatus

Abstract... V-1
Introduction... V-2
Materials and Methods.. V-3
Results and Discussion... V-4
References... V-10

CHAPTER VI. Degradation of Atrazine in Estuarine Water/Sediment Systems and Selected Soils

Acknowledgements... VI-ii
Abstract... VI-1
Introduction... VI-2
Materials and Methods.. VI-3
Results and Discussion... VI-6
References... VI-19

CHAPTER VII. Uptake and Photosynthetic Inhibition of Atrazine and its Degradation Products on Four Species of Submerged Vascular Plants

Acknowledgements... VII-ii
Abstract... VII-1
Introduction... VII-2
Materials and Methods.. VII-3
Results and Discussion... VII-4
References... VII-14

CHAPTER VIII. Effects of Nutrient Enrichment in Experimental Ponds Containing Submerged Vascular Plant Communities

Table of Contents.. VIII-ii
List of Figures.. VIII-iv
Introduction... VIII-1
Materials and Methods.. VIII-2
Results.. VIII-8
Discussion... VIII-30
References... VIII-54
Appendices... VIII-61

Page

CHAPTER IX. The Decline of Submerged Vascular Plants in Upper Chesapeake Bay: Summary of Results Concerning Possible Causes

Acknowledgements.. IX-ii
Abstract.. IX-1
Introduction.. IX-2
Approach to Problem..................................... IX-4
Herbicide Inputs, Fate and Phytotoxicity................ IX-4
Suspendable Sediments and SAV Light Responses........... IX-7
Nutrient Enrichment and Algal-SAV Relations............. IX-11
Synthesis of Findings and Implications.................. IX-14
References.. IX-19

SUMMARY AND CONCLUSIONS

This report presents results of research conducted at University of Maryland's Center for Environmental and Estuarine Studies concerning factors potentially involved in the decline of submerged aquatic vegetation (SAV) in upper Chesapeake Bay. This research examines three main factors in relation to SAV growth and production: agricultural herbicides; suspended sediments and associated light attenuation; nutrient enrichment and resulting algal growth and light attenuation. A few ongoing studies (e.g., Goldsborough 1983, Staver 1983) are not included here; however, in this discussion some reference is made to their findings which will be available soon. The results of other relevant studies are summarized elsewhere (Boynton et al. 1983; Wetzel et al. 1983).

Considerable effort was expended to investigate the potential importance of herbicides in contributing to the overall stress of the estuary's SAV populations (Chapters I-VII). This research emphasized two specific compounds. The first of these, atrazine, which is closely associated with corn crops, has been the most widely used herbicide in the region, while the second compound, linuron, is commonly employed in weed control for soybeans. Concentrations of these two herbicides were monitored in water and sediments throughout the upper bay over the period 1980-81 (Chapter I). A hierarchically designed stratified sampling scheme revealed typical aqueous concentrations of both compounds to be about 0-3 ppb in the main bay, 0-5 ppb in a major eastern shore tributary, and 0-40 ppb in a creek connecting a small estuarine cove to surrounding agricultural fields. Concentrations in the creek and small cove were measured at 1-4h intervals before, during and after all runoff events, and values above 5 ppb never persisted for more than 6-8 h. Atrazine concentrations associated with suspended or deposited sediments were less than 5 ppb for > 95% of samples and never exceeded 20 ppb.

Initial studies indicated a wide range of physiological and morphological responses of one common SAV species, Potamogeton perfoliatus, in response to herbicide treatment, including photosynthetic depression, stem elongation, reduction in stem weight per unit length, and increased chlorophyll a per unit leaf area (Chapter II). Several of these effects are analogous to observed adaptations of this and related species to reduced light intensity (e.g., Goldsborough 1983).

At atrazine or linuorn concentrations between 5-100 ppb significant photosynthetic inhibition was observed for both P. perfoliatus and Myriophyllum spicatum in microcosms, followed by strong recovery (toward untreated control plants) within 1-3 wk, eventhough herbicide levels remained within 5-10% of initial values throughout (Chapters II and III). Plant biomass decreased significantly after 5 wk of treatment at herbicide concentrations > 50 ppb for

P. perfoliatus and > 500 ppb for M. spciatum (Chapter III). Overall, the effects of the two herbicides were statistically identical, while some differences between plant species were observed (M. spicatum being more tolerant). Estimates of $I_{1.0}$ (herbicide concentration at which 1% loss of photosynthesis (P_a) is predicted) were 2-4 ppb for P. perfoliatus and 8-11 ppb for M. spicatum, and values of I_{50} (concentration for 50% loss of P_a) ranged from 45-55 ppb and 80-117 ppb respectively. Similar phytotoxicities were observed for Zannichellia palustris and Ruppia maritima (Chapter VII).

Rapid uptake of ^{14}C-labelled atrazine was demonstrated for P. perfoliatus, with equilibrium between internal and external concentrations being achieved within about 1 h (Chapter IV). A direct relation between atrazine uptake and photosynthetic depression was observed for this plant; however, disproportionately high apparent uptake at low external herbicide concentrations suggests a two-step uptake process with simple sorption (without inhibition of photosynthesis) dominating at low concentrations. Root uptake of atrazine appears to be of little importance for these plants. Initial photosynthetic recovery of atrazine-treated plants was effected by release of sorbed herbicide within 2 h after rinsing in atrazine-free water. Some (~ 5%) loss of photosynthesis was evident after 3 d of wash; however, this difference was not statistically significant. Short-term (2 h) experimental exposures to atrazine revealed reductions in P. perfoliatus photosynthesis which were similar to those observed over 2-6 wk in microcosms, with values of I_{50} being about 80 ppb.

Atrazine is readily sorbed to soil and sediment particles, with a partition coefficient (sorbed:aqueous) greater than 1.0 (Chapter V). However, the potential importance for plant uptake of atrazine sorbed to overlying sediments (resting on SAV leaves) seems to be remote. Experiments with ^{14}C-labelled atrazine showed negligible plant uptake of herbicide sorbed to soils concentrations of at about 120 ppb. In addition, the presence of epiphytic sediments significantly retarded leaf uptake of aqueous atrazine, although such sediments themselves inhibited photosynthesis presumably by attenuation of light and reduction of CO_2 utpake.

The degradation of ^{14}C-labelled atrazine was observed under simulated field conditions for upper and middle bay sediment-water systems and for two common agricultural soils in the Maryland coastal plain. The distributions of atrazine and two categories of metabolites or degradation products (hydroxyatrazine and dealkylated atrazine) were followed over an 80 d period. The half-life (time for 50% degradation to metabolites) for atrazine was markedly shorter for estuarine systems (15-20 d) than for soils (330-385 d). The accumulation of hydroxyatrazine in experimental estuarine water and sediments raised questions concerning the potential phytotoxicity of these compounds. Bioassay experiments were performed with 4 species of SAV to examine uptake and photosynthetic depression for ^{14}C-labelled atrazine and 3 metabolites (Chapter VII). Overall, the inhibitory effect of the metabolite, hydroxyatrazine, on plant photosynthesis was negligible compared to that for atrazine, with no significant inhibition even at 1500 ppb. Some significant loss of P_a was observed for deethylated atrazine at 500 ppb; however, this metabolite has a short half-life in the estuary, being similar to that for atrazine.

The effects of nutrient enrichment on algal (plankton and epiphytes)

growth and SAV production and abundance were investigated by fertilizing 8 (duplicates at 4 levels) experimental ponds (500 m^2) during June-August 1981 (Chapter VIII). These ponds, which were seeded with sediment, water and plants from the Choptank River estuary, were maintained in batch-mode for sequential periods of 7-10 d punctuated by complete exchange of water followed by retreatment prior to the next batch period. Maximum fertilization rates were typical of nutrient loading in areas of upper Chesapeake Bay receiving direct agricultural runoff. Nutrient concentrations in treated ponds were reduced rapidly to control levels within 1-3 d, and plant tissue nutrient contents were directly related to treatment. Initial growth of the two dominant SAV species (*P. perfoliatus* and *R. maritima*) was enhanced in fertilized ponds; however, plant abundance in August was inversely related to treatment, with SAV virtually eliminated at the highest dosage.

Planktonic and epiphytic algal biomass (as chlorophyll *a*) increased significantly with treatment. Light (PAR) attenuation by microalgae was sufficient to account for the reduction in SAV production and abundance in August. Epiphytic growth accounted for most of the light reduction although attenuation in the water column was also necessary to reduce PAR below plant compensation levels. Direct measurements of epiphyte effects on both PAR attenuation (by leaf scrapings in petri dishes) and plant photosynthesis (with ^{14}C-labelled bicarbonate) confirmed this relationship. Preliminary evidence suggests that a shortening of SAV growing season, as observed here in response to fertilization, may ultimately lead to decimation of these plant populations by disrupting plant reproduction. Light attenuation by microalgae and suspendable sediments may affect the normal balance between SAV production and respiration leading to premature flowering and/or insufficient translocation to underground propagules, both of which would reduce the viability of regrowth in the following spring. It is concluded that further research is needed to understand the reproductive capacities and strategies for these plants.

The results of 2 recent studies continuing this line of research are not included in this report but will be available soon. A second year (1982) of fertilization in the experimental ponds provided a more detailed examination of the nutrient-algal-SAV relationships (Staver 1983). Problems encountered in the batch-mode approach in 1981 were alleviated with a continuous flow system and more frequent treatment. In this 1982 study only 4 ponds were used, and SAV communities in these were essentially mono-specific stands of *P. perfoliatus*, thus eliminating the complicating problems of differential epiphytic colonization on 2 SAV species. The general patterns observed in 1981 were more pronounced and less equivocal in the 1982 research. Detailed studies of the responses and adaptations of *P. perfoliatus* to (high 100%, medium 34%, low 6%) light were also done in 1982 (Goldsborough 1983). Numerous morphological and physiological changes in this plant were observed in response to reduced (moderate and low) light, including stem elongation, increased pigmentation, increased specific leaf area, as well as increased initial slope for photosynthesis versus irradiance relations. Most of these adjustments appear to confer adaptive advantage on shaded plants; however, after 2 wk of exposure to low light significant reductions in stem density, flowering and underground reproductive propagules were observed.

A summary and synthesis of the research included in this report is pro-

vided in the final chapter (IX). Here, we consider the relative contributions of herbicide runoff, sediment loading and nutrient enrichment to the environmental stress experienced by SAV in upper Chesapeake Bay. Combining these research findings in a simple conceptual framework as well as a numerical simulation model suggested that the relative importance of effects on SAV associated with these 3 inputs is as follows, nutrients > sediments >> herbicides. A narrative scenario is developed to interpret spatial and temporal aspects of the SAV decline in light of this research, and potential resource management strategies are discussed.

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CHAPTER I
CONCENTRATIONS OF THE HERBICIDES ATRAZINE AND LINURON IN AGRICULTURAL DRAINAGE, TRIBUTARY ESTUARIES AND LITTORAL ZONES OF UPPER CHESAPEAKE BAY*

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INTRODUCTION

Although herbicides have provided effective weed control resulting in higher crop yields and are a necessity for modern no-till agriculture (Phillips et al. 1980), they may also have deletorious environmental effects upon non-target species (Galston 1979). Recently there has been concern that herbicide runoff from agricultural fields may have played a role in the decline of submerged aquatic vegetation (SAV) in Chesapeake Bay (Stevenson and Confer 1978). Early changes in SAV populations coincided with increased herbicide usage in the Chesapeake watershed in the 1960's (Bayley et al. 1978). During that period the principle herbicide was atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine) which was applied to large acreages of corn. Later, other herbicides such as linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea], were also used widely because of a shift to soybeans which were planted in rotation with corn every other year.

Previous reports of the presence of atrazine residues in aquatic systems receiving runoff from agricultural areas showed concentrations could reach 20 $\mu g\ \ell^{-1}$ (Waldron 1974, Richard et al. 1975, Miur et al. 1978, Schepers et al. 1978, Frank et al. 1979, Roberts et al. 1970). However, these studies were all done in freshwater systems which differ in many ways from estuaries such as Chesapeake Bay. The possibility of high herbicide levels in Chesapeake Bay waters was first suggested by Correll et al. (1978) who reported dissolved atrazine and linuron concentrations in the range of 1-10 ppb in several upper bay tributaries and up to the ppm range in sediments. However subsequent measurements by Austin (see Stevenson and Confer, 1978) and Newby et al. (1978) in surface waters of the open bay showed concentrations of atrazine only as high as 1.0 $\mu g\ \ell^{-1}$ and concentrations declined rapidly in a non-conservative manner to undetectable levels ($<0.1\ \mu g\ \ell^{-1}$) in a seaward direction. Similarly, Zahnow and Riggleman (1980) reported that concentrations of the pheuylurea herbicide linuron were non-detectable ($<.01\ \mu g\ l^{-1}$) in either the water column or sediments in several tributary rivers of the bay, including the Choptank and Rhode River estuaries. The variability between these studies may have resulted for a number of reasons, including: the proximity of sampling times to run-off events; the persistance of different herbicides in estuarine waters; the location of sampling stations relative to herbicide source area (littoral zone vs. open bay waters); and differences in herbicide loadings due to annual variations in precipitation and resultant runoff.

In view of these uncertainties, and the limited amount of herbicide data available, we conducted a three-phase investigation of selected herbicide (atrazine and linuron) concentrations in the upper Chesapeake Bay (Maryland portion of bay). The first phase of this investigation documented herbicide concentrations in water and sediments at 23 littoral zone sites throughout the upper bay where SAV communities were: (1) still present; (2) now absent but had been present in the recent past or; (3) had not been present for at least two decades. The second phase involved monthly measurements of herbicide concentrations along the longitudinal axis of a major upper-bay tributary (Choptank River estuary) receiving drainage from an agriculturally dominated watershed. The third phase involved detailed monitoring of herbicide discharge from an experimental agricultural watershed where application rates were known. Our objectives were to develop a preliminary description of the time-space characteristics of herbicide concentrations in the upper bay and to relate these field observations to *in situ* distributions of SAV and to experimentally derived relationships between SAV growth and herbicide dose.

METHODS

Sampling Design

Herbicide concentrations in water (dissolved) and sediments were monitored at twenty-three shallow water (0.5 - 1.0 m depth) sites in the upper Chesapeake Bay (Fig. 1; Table 1) on 4 occasions (23-28 April; 11-16 June; 24-29 July; 15-22 September in 1980). Sampling areas were chosen along the open bay shoreline throughout the upper bay, in tributary rivers and in hydrodynamically quiescent coves to provide a representative sampling of SAV habitats. In each habitat area several stations were identified and included: (1) those having SAV communities (Status I as indicated by the 1978 aerial surveys of Anderson and Macomber, 1979); (2) sites at which SAV had been present prior to 1969 (Stotts, pers. comm) but had not been present within the last decade (Status III); and locations which had not supported SAV communities at least since 1959-61 (Status IV). During the present study there were 3 sites where vegetation had been present in 1978 but failed to appear in 1980; these stations were designed as being Status II.

Atrazine and linuron were also measured monthly from April through September at 4 to 7 locations along the longitudinal axis of the Choptank River estuary, a major eastern shore tributary of Chesapeake Bay (Fig. 2). Sampling sites were selected based on salinity (S) conditions in the estuary during each cruise such that there was equal increment in surface salinity among stations from tidal-freshwater (S=1 ppt) to the bay (S=9-17 ppt). In addition, one station was routinely sampled at a site mid-way from the main channel and littoral zone station SI-14 (Todds Cove). A single water sample was collected at 0.5 m at each station for dissolved residue analyses. In addition one sediment grab was also collected at each station and a sub-sample of surficial material (top 5 cm) was analyzed for residues. On an occasional basis, 10-20ℓ of near-surface water was filtered and the particulate fraction analyzed for residues.

A controlled agricultural watershed (Fig. 3) was monitored to estimate herbicide delivery to the Choptank River estuary. Runoff and tidal influences were monitored using a 1 m width Parshell flume (Lomax et al. 1979).

Downstream from the flume, four additional stations were also sampled, the last being adjacent to the Choptank River. Runoff events were monitored prior to application of agricultural fertilizers and herbicides, immediately following application and during the summer period after the emergence of field crops. Herbicide runoff was also monitored on a weekly basis during 1981 at the U.S. Geological Survey gauging station at Beaverdam (located on Kings Creek which enters the Choptank River 55.5 km upstream of the mouth) which drains a larger watershed.

All water samples for herbicide analyses were collected in pre-cleaned and solvent washed 2 liter glass jars with aluminum foil-lined caps. Immediately after sampling, a 1 liter aliquot of each sample was filtered through precombusted Whatman GFC glass fiber filters (1.2 μm) and pumped through a pre-cleaned SEP-PAK C_{18} cartridge at a rate of 3 mℓ min^{-1} to concentrate herbicide residues. After the sample was pumped, the bottle was washed down with 50 mℓ of distilled water and was also pumped through the SEP-PAK. Finally, 15 mℓ of distilled water was pumped through the SEP-PAK to remove salts. The SEP-PAK cartridges were placed in individual pre-cleaned and solvent washed 50 mℓ capped vials and frozen at -20 C until analyzed in the laboratory. The balance of each water sample was refrigerated at 4 C as a reserve sample until all analyses are completed on the pumped aliquots.

Sediment samples for herbicide analyses were collected using a stainless steel coring device (4 cm diameter x 60 cm). Triplicate 10 to 15 cm cores were collected and the top 5 cm were subsampled in the field for analysis. The sediment cores (1 kg wet sediment per replicate) were stored in pre-cleaned and solvent washed one quart glass jars with aluminum foil-lined caps and frozen at -40 C until analyzed. At selected stations water samples were collected and analyzed for pariculate bound herbicides. A 20 liter sample was filtered in the field through precombusted Whatman GFC glass fiber filters and the filters wrapped in aluminum foil and frozen at -40 C until analyzed.

Herbicide Analysis

All glassware and apparatus used in herbicide extraction and clean-up procedures were washed with glass distilled deionized water and pesticide grade methanol. The extraction of frozen sediment was achieved by weighing the frozen material in a tared, pre-extracted soxhlet thimble. The samples were extracted in a Soxhlet apparatus for 24 hours with 200 mℓ of distilled-in-glass (DIG) methanol (Burdick and Jackson, Muskegon, MI). Following extraction the samples were dried to constant weight to obtain the dry weight of material extracted.

The methanol extracts were dried over anhydrous sodium sulfate, evaporated in a rotary vacuum evaporator to approximately 10 mℓ of DIG methanol for analysis. Some samples required clean-up prior to analysis. When this was necessary, the entire 1 mℓ sample was passed through a preparative reverse-phase liquid chromatographic column (Bondapak C_{18}) using a solvent system of 50:50 methanol:water solvent system. The fraction(s) for atrazine and/or linuron were collected, evaporated under nitrogen and taken up to 1 mℓ of DIG methanol for quantitative analysis.

The SEP-PAK containing water column residues were eluted with 5 mℓ of DIG methanol and the eluent was evaporated under a stream of nitrogen and taken up in 1 mℓ of DIG methanol containing a known quantity of an internal standard (diphenylamine) for a quantitative analysis.

Atrazine residues were quantified using a Hewlett-Packard 5840 gas chromatograph equipped with a 2 mm ID x 2 M all-glass column packed with 10 percent Carbowax 20 M on 80/100 Supelcoport. The column was maintained at a temperature of 160 C and the injector and detector were held at 225 C and 300 C, respectively. An alkali-flame nitrogen-phosphorus specific detector was used to measure the amount of atrazine and internal standard in a 4 μℓ sample. Quantification was achieved by comparing the atrazine/internal standard ratio (AT/IS) to a calibration curve of AT/IS vs. atrazine concentration.

Linuron residues were analyzed using a Waters Associates 6000A liquid chromatograph equipped with a μ Bondapak-Phenyl (Trade name) column, a model 440 fixed wavelength ultraviolet detector (254 μm), a Perkin-Elmer M-2 electronic integrator and a Linear 159 chart recorder. Twenty microliter samples were introduced onto the column using a Rheodyne 7125 injection valve and eluted using an isocratic solvent system of 35 percent acetonitrile/water. Areas under the sample peaks were integrated and quantified by comparison with a calibration curve.

Quality Assurance Program for Analysis of Herbicides

As an integral part of the analytical program for this study, a quality assurance (QA) program was developed which included both frequent internal checks and periodic external checks on the precision and accuracy of the analytical methods employed for trace analyses of herbicides in water, suspended particulates and sediments of Chesapeake Bay.

The internal program for quality assurance started in the field and included the establishment of sampling protocols and storage procedures which ensured that herbicide residues present in the samples at the time of sampling would not degrade or volatilize from the samples. During the first year of this study storage experiments were performed both internally and as part of an external QA audit to assure that the integrity of the samples was preserved. These studies indicated that no detectable losses of sample at environmentally realistic levels (1-2 ppb) occurred under the conditions used. The details of the sampling and storage techniques are presented in the methods section of this report.

With the large number of samples being collected and analyzed, record keeping was recognized as an important component of our QA program. The following steps were taken to insure continuity of sample identification and data reporting.

1. All samples were labeled at sampling with the date, site of origin and sample type. These data were also entered in a continuous logbook at the time of sampling. Field work sheets documenting the sampling data and site

informaton were also filled out at each station.

2. A second book contained all of the written laboratory methods employed for sampling, storage, extraction, concentration and analysis.

3. All weights and measures used during the preparation of the samples for analyses were recorded in a laboratory record book along with the appropriate sample identification information.

4. All analytical data relating to the quantitation of the herbicide residue were kept on the chromatograms.

This last step was the most convenient for us since: 1) the integration data and quantifications were printed on the chromatograms in one case or were printed on a tape and stapled to the corresponding chromatogram and 2) this allowed us to keep the standard chromatograms and sample chromatograms together for future reference. Each working standard curve used for quantitation was also retained in the file. Our methods were set up such that a minimum of calculation was necessary. Peak areas or area/area ratios (internal standard/sample) were divided by the slope of the working calibration curve and corrected for concentration factors or extraction weights. Quantitation data summaries for logical groups of samples (i.e. 1979 field H_2O samples; 1980 linuron H_2O samples; etc.) were then prepared.

At the analysis stage, the internal QA program was centered upon maintaining a consistantly high level of analytical results. The program was designed to insure: 1) quantitative recovery of herbicide residues from water and sediment samples, and 2) accurate, reproducible and precise determination of the concentrations of herbicide residues in water, suspended particulates and sediment extracts. The program included the following steps:

1. Preparation of fresh standards from 99.5+% pure analytical grade compounds and intercomparisons of old standards with new standards every two weeks.

2. Daily three-point checks of working standard curves and recalibration of instruments before sample analysis and preparation of new standard curves when instrument parameters were changed (i.e., new column, new detector bead, etc.).

3. Method blanks (water, solvent, SEP-Pack) were run with each batch of samples. All blanks were below our detection limit.

4. Blind samples with known residues were periodically inserted in the sample analysis schedule. No sample varied more than 10% from the known amount.

5. One in 10-12 samples was analyzed in duplicate. No variations of more than 10% were observed.

6. All samples were injected in duplicate. If results varied by more than 5%, the samples were reanalyzed.

7. Samples which were analyzed were reanalyzed with a known spike. Recoveries of 90-100% were considered acceptable.

8. Mass spectral confirmation using a three ion/selected ion monitoring technique was employed on field samples with significant residues (>0.6 ppb) to confirm the identity of the herbicide residue and to cross-check the quantitation.

In addition to the internal program for QA the laboratory was evaluated three times by external QA auditors. In each case, blinded samples were received, spiked in duplicate into estuarine water, and analyzed by the normal laboratory protocols. In each case, the results of our analyses were within the acceptable limits of accuracy, reproducibility and precision. (Appendix Tables 10 and 11).

The following methodological parameters were determined in these external audits.

	ATRAZINE	LINURON
Accuracy*	± 15	±15%
Reproducibility (1 ppb)	± 3.0%	±2.9%
Precision	± 0.016	±0.006
Detection Limit	0.05 ppb (water) 0.1 ppb (sediment)	0.05 ppb (water) 0.1 ppb (sediment)

* includes extraction efficiency

As stated earlier, the first external QA audit included a check on sample storage procedures. No significant losses were observed using our storage protocols.

RESULTS AND DISCUSSION

Upper Bay Littoral Zone Investigations

Measurements of dissolved atrazine and linuron concentrations, as well as SAV biomass measurements, associated with the littoral zone investigations are listed in Appendix Table I. During the April, June, July and September cruises, atrazine concentrations ranged from $\leq$0.05-0.3 ppb, $\leq$0.05-1.14 ppb, $\leq$0.05-0.4 ppb and $\leq$0.05-0.93 ppb, respectively. Maximum dissolved atrazine concentrations were consistently less than 2 ppb and the majority of samples yielded concentrations below the detection limit (0.05 ppb). During the April and June sampling periods linuron residues were below the detection limit (0.05 ppb) at all 23 stations while in July and September concentrations ranged from $\leq$0.05-1.3 ppb and $\leq$0.05-0.75 ppb, respectively. The lack of detectable linuron residues in April and June was attributed to the fact that large quantities of this herbicide are usually not applied until July when soybeans are planted.

To provide a broad view of dissolved herbicide distribution in the upper bay we have summarized our data by averaging concentration measurements from similar geographical areas during each of the 4 sampling periods (Table 2). Atrazine residues above detectable concentrations were observed earlier in the year and within more geographical locations in the bay than was the case for linuron. For example, dissolved atrazine $\geq$0.1 ppb occurred at 32-36% of our stations from April-July while such concentrations of linuron were exceeded at only 13-20% of stations in July and September. Finally, for both herbicides, averaged concentrations were generally higher in either the upper bay or tidal fresh tributary areas than in other geographical groupings, although some exceptions occurred (e.g. mid and lower bay in April).

While averaged herbicide concentration data is useful in providing a general view of conditions in the upper bay, the time-space occurrence of maximum concentrations is also important (Table 3) particularly since it appears that short-term (1 hr) exposure of seagrasses to atrazine can depress photosynthetic rates (Jones et al., 1983). In April maximum dissolved atrazine concentrations were relatively low in all areas ($\leq$0.05-0.30 ppb) but tended to be somewhat higher in the mid and lower bay groups (SI's 1 and 24). However, maximum concentrations in June, July and September tended to occur in the upper bay and/or tidal fresh tributaries, paralleling the trend in the averaged data presented in Table 2. For reasons that are not clear at this time, stations in the vicinity of the Patuxent River estuary (SI's 1, 2 and 27) consistently had the highest concentration in the mid-bay region and in the tidal freshwater category on 2 of 4 occasions (SI-27). Except that detectable concentrations of dissolved linuron were not observed in the April and June surveys, the spatial pattern of maximum concentrations closely paralleled that observed for atrazine.

Sediment concentrations of atrazine and linuron were measured at each of the 23 littoral zone sites during each survey. Detectable concentrations are given in Table 4 and the results of all analyses are contained in Appendix 2. Detectable concentrations in surficial sediments were generally lower and much less frequently observed than in the water column. Detectable levels of atrazine (>0.1 μg kg^{-1}) were found in bottom sediments only 5 times during the entire survey (total sample number = 82) and none exceeded 1.0 μg kg-1. Atrazine residues were most frequently encountered in June and all occurred in the upper bay (SI's 5 and 7) or tidal-fresh tributaries (SI-18). Linuron concentrations in sediments exceeded limits of detectability at only 1 site (SI-23; 0.51 μg kg^{-1}) near the town of Crisfield in September. It appears that little of either atrazine or linuron remains associated with sediments in the estuary. Cunningham (1980) showed that over 90% of atrazine adsorbed to sediment is released when placed in estuarine water. In addition, Jones et al. (1982) reported that atrazine in the water column degrades rapidly with a half-life of 1-2 weeks, suggesting that estuarine concentrations may be ephemeral. Linuron has an even faster degredation rate in estuarine waters (Means et al., 1983) which may account for its even lower frequency of occurrance in the study area.

Choptank River Longitudinal Survey, 1980

To obtain a more detailed characterization of herbicide distributions in one area of the bay, monthly samples were taken along the longitudinal axis (channel stations) of the Choptank River estuary, the watershed of which is agriculturally dominated. Station locations were given in Figure 2, and all measurements of dissolved atrazine and linuron concentrations are listed in Appendix Table 3 and summarized in Figure 4. The general pattern that emerged for both compounds was one in which concentrations were near or below detection levels in the early spring (April and May) and late summer (September) and in the range of $\leq$0.05-1.0 ppb during the months of June, July and August. There was some indication that highest residue concentrations occurred in the tidal-fresh region as might be expected since the ratio of river volume to drainage area is minimal and hence the potential for dilution is limited. However, on several occasions elevated concentrations were found in other regions of the estuary suggesting that inputs from adjacent agricultural fields might be of sufficient magnitude to elevate residue concentrations in the mainstem channel areas. In any case, dissolved concentrations were generally below 1.0 ppb throughout the study area and never exceeded 2.0 ppb. Of the 38 stations sampled, 53% exhibited residue concentration of atrazine below detection levels and only 26% had concentrations $\geq$.10 ppb. Only 16% of the stations sampled showed significant concentrations of linuron.

Consistent with the results of the upper bay littoral zone survey, sediment concentrations of atrazine and linuron were even lower and detectable concentrations ($\geq$0.1 μg kg^{-1}) were rarely encountered (Appendix Table 4). In fact, of the 38 stations sampled only 2 had detectable concentrations of atrazine or linuron. While considerably fewer samples were analyzed for herbicide residues associated with the suspended particulate fraction, we did not detect significant concentrations ($\geq$0.1 μg kg^{-1}) in any of samples analyzed (Appendix Table 5).

Agricultural Runoff Studies

In 1980 relatively low spring precipitation caused very low superficial water tables in the Choptank River region where our intensive runoff studies were centered. The silty-clay Mattapax soils of this region have a high moisture holding capacity and can absorb large quantities of water when dry. As a result of the precipitation-soil conditions evidenced in 1980, there was virtually no measurable runoff prior to July and hence it is doubtful that data collected during this period are representative of more normal years. In any case, concentrations of dissolved atrazine and linuron measured during 1980 at several locations on the Horn Point Experimental watershed are listed in Appendix Table 6 (see Fig. 3 for station locations). During the monitoring period, concentrations of linuron in agricultural base-flow drainage ranged from below our level of detection ($\leq$0.05 ppb) to 2.61 ppb in a small embayment (pond) adjacent to the Choptank River. Peak atrazine concentrations ranged from 8.2 ppb in the flume to 18.1 ppb immediately downstream adjacent to a cornfield. Atrazine concentrations were more frequently in excess of 1.0 ppb earlier in the year, suggesting that even when baseflow runoff is the predominant means of transport, maximum concentrations still occur shortly after application of these compounds. During dry conditions we also found that elevated herbicide concentrations were largely restricted to drainage ditches connected to agricultural fields. As indicated in Appendix Table 6 all samples taken from the Choptank adjacent to agricultural fields exhibited residue concentrations below detection levels, even when appreciable concentrations were observed in drainage creeks. Apparently degradation rates for these compounds are sufficiently rapid (Jones et al. 1982) coupled with dilution in estuarine waters such that elevated concentrations were not observed in open waters of the Choptank adjacent to the experimental watershed in 1980.

Of the 67 determinations of atrazine and linuron residues associated with suspended particulates in the water column, only one sample exhibited a significant concentration (Appendix Table 7). Similarly, atrazine and linuron concentrations in soils collected from cropland and drainage chennels were generally below detection levels ($\leq$0.1 μgkg^{-1}) and never exceeded 1.2 ppb (Appendix Table 8). Hence it appears that during 1980 there was little mass transfer of herbicide in any form (dissolved or adsorbed) between fields and estuary nor was there a significant accumulation of residues in cropland or aquatic sediments.

However, in 1981 precipitation in the spring and early summer months was near average and runoff events at the Horn Point Experimental watershed were recorded up until mid-summer when a protracted dry period began. Also, flows at Beaver Dam (Fig. 2) measured at the U.S. Geological Survey station on Md. Rt. 331 were substantial following spring planting.

Atrazine is usually applied at a rate of 2.2 kg ha^{-1} during final disking and seeding operations in conventional tillage of corn used by over 90% of the farmers in the Choptank region (R. Wade, Agricultural Extension Agent, Dorchester Co., MD). Normally, planting begins in late April on excessively drained sandy soils (Gailstown) in this area; followed by planting in moderately well drained soils (e.g. sassafrass) and finally proceeds to heavy clay soils (e.g. Mattapex) in May when evapotranspiration dries up excessive moisture. Therefore in a large (1500 ha) watershed such as that around Beaverdam Branch, there is a progression of application on various soils over a number of weeks, which began in late April in 1981.

Despite its much larger size the Beaverdam watershed behaved similarly to the Horn Point Experimental watershed except that concentrations were detectable a little earlier than at Horn Point due to earlier application. However, since the peak concentrations at Beaverdam was lower (9 ppb) during the intensive runoff period of May 11-15 (Figs. 5a and 5c) total delivery of atrazine to the Choptank River was almost identical per watershed (Appendix

Table 9). During the highest runoff month (May) an estimated 4.6 kg atrazine was transported from the watershed, while only 0.2 kg and 0.07 kg were transported in June and July, respectively. Therefore the total 1981 atrazine leakage rate was in the range of Horn Point about 0.6% of the input. This latter loading was derived from an estimate of corn acreage in the Beaverdam watershed from a small plane and the recommended rate of application for this area (2.2 kg ha^{-1}).

One of the reasons for this relatively low rate of atrazine leakage may be that delivery occurred after the study period but Wauchope and Leonard's (1980) formula suggests little leakage that late in the season. A more likely reason is that relatively low pH which ranged from 4.5 to 6.8 during this period reduced atrazine mobility. Runoff losses of atrazine have been reported to be much lower in unlimed soils and leaching increases with increased pH (Gaynor and Volk, 1981). Another important factor is that the relatively flat eastern shore landscape may preclude as much herbicide runoff as that found on the western shore (1%) by Wu (1980). Therefore, herbicide application on Maryland's eastern shore may present potential fewer environmental problems than originally anticipated by Correll et al. (1977).

In contrast to the extended period of herbicide application at the Beaverdam watershed, 18 ha of corn was planted in only two days at the Horn Point Experimental watershed beginning on May 4, 1981. Initial atrazine concentration in ditches on this date was undetectable, demonstrating little spray drift during application (Appendix Table 9).

A week after application, the largest runoff events of 1981 resulted from a series of thunderstorms which passed over the region (Fig. 5c). At Horn Point, 12.2 x 10^3 m^3 of water passed through the flume in the agricultural field over the period of May 11-15. Atrazine concentrations ranged from 1.6 to a peak of 13.7 ppb in samples taken over this period. An estimated 197 g atrazine was lost downstream or about 0.5% of the total application (39.6 kg). It appears that runoff throughout the basin was significant enough to increase atrazine concentrations in the estuary proper (Fig. 5b) over those observed in 1980 (Fig. 4).

Although there were several small precipitation events later in the month, none was large enough to promote runoff. In succeeding months, due to declining atrazine concentrations, much less residue was delivered to the Bay. In June an estimated 34 g was lost and 16 g in July. The total delivery during this 3 month period accounted for 0.6% of the total application. Since a prolonged drought period began in late July, very little further herbicide leakage occurred in 1981 from the Horn Point watershed. Also, although soybeans were planted in July very low concentrations (<2 ppb) of linuron plus little flow of water produced unestimatable low leakage of linuron. We discuss the temporal coupling of runoff and herbicide application in more detail in a later section of this report.

SAV-Herbicide Relationships

We had anticipated finding SAV communities at 10 of the 23 littoral zone stations sampled in 1980 based on the results of the 1978 aerial surveys of Anderson and Macomber (1979). However, at 3 sites (SI's 15, 20 and 23) aboveground biomass never appeared during 1980 and at all upper bay locations where SAV was present in 1980 (SI's 4, 9 and 28), biomass disappeared prior to the September sampling period. In addition, qualitative observations taken since 1980 indicate that SAV has declined at other of our stations including SI's 10, 13 and 14 (Staver, pers. comm.; Kaumeyer, pers comm.). In contrast, SAV communities around Smith Island (SI-21) in the lower bay appeared robust in late 1982 (Kemp, pers comm.). It appears that some declines in distribution and abundance have occurred post-1978 and continued through 1982, at least at the stations we sampled. The results of SAV biomass sampling are listed in Appendix Table 1 and summarized in Table 5. In general SAV appeared to be more ephemeral in the upper bay region than in the mid and lower bay although the highest mean biomass observation was obtained at a location in the upper bay (SI-28). With few exceptions (e.g. SI-28 in June; SI-14 in July) SAV standing stocks were low compared to those observed in other SAV studies in the bay (Kaumeyer et al., 1980; Wetzel et al., 1980).

To explore our data for possible relationships between herbicides and SAV abundance, dissolved atrazine and linuron concentrations measured during field surveys were plotted against SAV biomass estimates (Fig. 6). The resultant scatter plots suggest little in the way of strong patterns except that a broad range in SAV biomass was associated with herbicide concentrations below our level of detection. An alternative approach is to consider SAV status (i.e. present, early disappearance in 1980, not present since 1969 and not present since 1959-61) relative to observed herbicide concentrations. Specifically, we calculated the percent occurrance of atrazine above levels of detection for all four bay surveys for each SAV status group. Results indicated that atrazine concentrations were above detection levels, 45%, 50%, 42% and 66% of measurements in Status I, Ia (a sub-class of I where SAV disappeared early in 1980), III and IV areas, respectively. Again, strong trends did not emerge although there was some indication that detectable herbicides were more frequently associated with those areas where SAV had not been observed since the 1959-61 period and in areas where SAV disappeared early in the 1980 growing season. The frequency of atrazine occurrance was similar in areas with normal SAV in 1980 and in those areas where SAV had not been observed since 1969. However, if the above calculation is done using 0.5 ppb as a criteria for significant herbicide presence, a stronger pattern appears wherein this criteria is equalled or exceeded in 7%, 20%, 13% and 42% of observations in areas having SAV Status I, Ia, III and IV, respectively. While this is more suggestive of herbicide influence the pattern may be the result of some other factor co-varying with herbicide presence (nutrient and/or sediment effects on light attenuation). In the case of SAV it has been postulated that nutrient enrichment and light attenuation by sediments, in addition to herbicides, may be stressing or limiting the distribution of these communities in the bay (Stevenson and Confer, 1978). The results of other experiments and field studies conducted as part of our overall SAV program were syntheized into an ecosystem simulation model, the results of which suggested the relative potential SAV stress of 3 factors to be nutrients>sediments>herbicides (Kemp et al., 1983).

While these studies indicated a secondary role for herbicides as an influence on SAV distributions in the upper Chesapeake, it is not possible to completely discount herbicides as a factor in some areas of the bay for several reasons. For example, in the field studies reported here our sampling schedule was sufficiently discontinuous (6 wk intervals) such that we could not consistently detect short-term elevations or depressions in herbicide concentration due to runoff events in all littoral zones of the bay and hence our herbicide values only represent instantaneous concentrations. However, elevated, but apparently ephemeral, herbicide concentrations occur and may represent a stress to SAV in some areas of the bay. Jones et al. (1982), for example, has shown that SAV photosynthesis was depressed even when exposure times were short (1 hr) and recovery took 4-8 hr in herbicide-free medium. In the course of experimental dose-response studies (Kemp et al., 1983) we did not test for the cumulative effects of repeated pulse exposures of SAV to herbicides and thus the effects of this type of exposure remain unknown. Additionally, the frequency of field measurements of herbicides were not sufficiently intense to determine the maximum levels of herbicide to which natural SAV communities were exposed.

An alternative procedure for evaluating possible impacts of herbicides on SAV is to compare in situ herbicide concentrations with the results of herbicide dose-response experiments conducted in conjunction with this study. A summary of the results of laboratory work reported by Kemp et al. (1983) is presented in Fig. 7. Herbicide concentrations causing 50% inhibition (I_{50}) of SAV net photosynthesis (P_a) ranged between 55-117 ppb and 45-80 ppb for atrazine and linuron, respectively. Concentrations causing incipient (1%) inhibition of P_a ranged from 2-11 ppb, consistantly higher than concentrations observed in littoral zones throughout the upper bay. Values for $I_{1.0}$ and I_{50} for SAV biomass were similar to those reported for macrophyte photosynthesis.

As indicated earlier in this report, maximum concentrations of atrazine and linuron approached 50 and 20 ppb, respectively, during a strong runoff event in May, 1981. More frequently concentrations were in the range of 1-10 ppb and existed for only short periods of time (2-8 h) being rapidly dissipated by dilution and degradation. Although some species may be more or less sensitive to herbicide stress than those we studied (e.g. Walsh et al., 1982; Correll and Wu, 1982) our in situ field observations of herbicide concentration coupled with laboratory dose-response work suggests that while herbicides may stress plants in some estuarine areas it is difficult to conclude that these compounds were primarily responsible for the bay-wide decline of SAV communities.

Annual Variability in Herbicide Concentrations

In this section we summarize some observations concerning the potential for significant annual variations in herbicide concentrations in the bay. Necessarily, much of what can be said is circumstantial but may still be useful in judging the representativeness of the data collected in 1980 and 1981.

In Fig. 8 we have summarized several features of freshwater flow from the

Susquehanna River for purposes of placing the results of the 1980 studies in perspective relative to this potential herbicide source. Considerable variation was evident among annual average flows as well as intra-annual flows between 1976-1982 ranging from about 52,000 cfs in 1979 to about 29,000 cfs in 1980 (Fig. 8). In this view 1980 was a low-flow year, as was 1981 and 1982.

Concentrations of atrazine detected in the Susquehanna River at Conowingo Dam (U.S. Geological Survey, 1982) were plotted against river flow and are shown in Fig. 9. With few exceptions detectable herbicide residues were associated with low river flow. Of the 14 occasions when detectable residues were observed, 12 occurred at river discharge rates below 40,000 cfs and the majority occurred at flows less than 20,000 cfs. Most of these lower flows occurred during May-September suggesting that herbicides entering the bay were largely derived from compounds applied to croplands in the spring or summer of the same year. The lack of detectable residues during the winter-spring freshet further supports this contention. Finally, there was little if any pattern in atrazine concentration among the years for which data were available, even though there were considerable differences in river discharge characteristics. In general, concentrations ranged from $\leq$0.1 ppb to 1.2 ppb, with values commonly in the range of 0.2-0.6 ppb between 1978 and 1981.

Atrazine concentrations measured at upper bay stations (e.g. SI's 4, 5, 6, 7, 8 and 28) in 1980 were generally comparable to those observed in discharge over the period 1978-1981. In fact, atrazine and linuron concentrations along the mainstem of the bay in 1977 and 1980 were generally comparable (Fig. 10). From the data available, it seems reasonable to tentatively conclude that dissolved concentrations of atrazine in the upper bay and along the mainstem are generally below 1 ppb and do not exhibit strong annual variabilities.

However the situation may be somewhat different in tributary rivers. In areas dominated by agriculture it appears that higher (10 ppb) herbicide concentrations can occur, even if only for brief periods of time. These events appear to be related more to local rainfall patterns and herbicide application times than to more general features of climate and river flow. Kemp et al. (1981) summarized atrazine runoff data reported in several studies and found that the total amount of residue transported into waterbodies was a function of the timing of the first runoff event after herbicide application; the longer the time interval between application and initial runoff event the lower the prcentage of applied herbicide which was removed. Wauchope and Leonard (1980) found an exponential decrease in concentration of atrazine with increasing time interval between application and initial runoff event. These results are consistent with those reported earlier in this paper. Since a strong runoff event occurred at the experimental watershed at Horn Point only 3 days after herbicide application in 1981, resultant concentrations may approximate maximum values to be expected in such tributary rivers (Fig. 5b). In any case, it appears that considerable short-term variability is to be expected in herbicide concentrations in waters derived from agricultural areas and tributary waters due to the interaction of application date and subsequent runoff events and the limited dilution potential of small creeks and rivers. Because of these transient events, it may well be worth investigating the cummulative response of SAV to repeated short-term exposure to elevated herbicide concentrations.

LITERATURE CITED

Anderson, R.R. and R.T. Macomber. 1980. Distribution of submerged vascular plants, Chesapeake Bay, Maryland. Rept. to USEPA, Chesapeake Bay Program by Aero Eco, Reston, VA.

Bayley, S., V.D. Stotts, P.F. Springer and J. Steenis. 1978. Changes in submerged aquatic macrophyte populations at the head of the Chesapeake Bay, 1958-1975. Estuarines 1:74-85.

Correll, D.L., T. Wu, J.W. Pierce and M.A. Faust. 1977. Rural non-point pollution studies in Maryland (non-point pollution studies on agricultural land use types prevalent in the Coastal Plain zone of Maryland). EPA 904/9-77-001. Washington, DC. 361 p.

Correll, D.L., J.W. Pierce and T.L. Wu. 1978. Herbicides and submerged plants in Chesapeake Bay, p. 858-877. In: Am. Soc. Civil Engineering. Coastal Zone-78.

Correll, D.L. and T.L. Wu. 1982. Atrazine toxicity to submerged vascular plants in simulated estuarine microcosms. Aquat. Bot. 14:151-158.

Cunningham, J.J. 1980. Effects of herbicide stress on the structure and metabolism of the submerged macrophyte, Potamogeton perfoliatus, in estuarine microcosms. M.S. Thesis, Univ. MD, College Park, MD.

Frank, R. and G.J. Sirons. 1979. Atrazine: Its use in corn production and its losses to stream waters in Southern Ontario, 1975-1977. The Science of the Total Environment. 12:223-239.

Galston, A. 1979. Herbicides: a mixed blessing. Bioscience 29:85-90.

Gaynor, J.D. and V.V. Volk. 1981. Runoff losses of atrazine and terbutryn from unlimed and limed soils. Environ. Sci. and Technol. 15:440-443.

Jones, T.W., W.M. Kemp, J.C. Stevenson and J.C. Means. 1982. Degradation of atrazine in estuarine water/sediment systems and selected soils. J. Environ. Qual. (In press).

Jones, T.W., W.M. Kemp. P.S. Estes and J.C. Stevenson. 1983. Atrazine uptake, phytotoxicity, release and short-term recovery for the submerged aquatic plant, Potamogeton perfoliatus. horn Pt. Environ. Laboratories, Box 775, Cambridge, MD.

Kaumeyer, K., W.R. Boynton, L. Lubbers, K. Staver, S. Bunker, W.M. Kemp and J.C. Means. 1981. Metabolism and biomass of submerged macrophyte communities in northern Chesapeake Bay, p. 353-400. In: Kemp et al. (eds). Submerged Aquatic Vegetation in Chesapeake Bay: It's Ecological Role in the Bay Ecosystems and Factors Leading to Its Decline. Horn Pt. Environ. Laboratories, Box 775, Cambridge, MD.

Kemp, W.M., J.C. Means, T.W. Jones and J.C. Stevenson. 1982. herbicides in Chesapeake Bay and their effects on submerged aquatic vegetation: A synthesis of research supported by U.S.E.P.A., Chesapeake Bay Program, p. 503-567. In: Chesapeake Bay Program Technical Studies: A Synthesis. U.S.E.P.A., Washington, DC.

Kemp, W.M., W.R. Boynton, J.C. Stevenson, R.R. Twilley and J.C. Means. 1983. The decline of submerged vascular plants in Chesapeake Bay: Summary of results concerning possible causes. Mar. Tech. J. (In press).

Lomax, K., J.C. Stevenson, M.S. Christy and J.R. Todd. 1979. Nonpoint pollution data collection problems on low flat coastal topography, p. 46-52. In: K. Flynn (ed.) Nonpoint Pollution Control - tools and techniques for the future. Interstate Commission on the Potomac River Basin, Rockville, MD.

Means, J.C., Wijayarante and W.R. Boynton. 1983. Fate and transport of selected pesticides in estuarine environments: Implications for ocean pollution. Can. J. Fish. Aquat. Sci. (In press).

Muir, D.C.G., J.Y. Yoo and B.E. Baker. 1978. Residues of atrazine and N-deethylated atrazine in water from five agricultural in Quebec, Arch. Env. Contam. Toxicol. 7:221-235.

Newby, L.C., R.A. Kahrs, K. Adams and M. Szolics. 1978. Atrazine residues in the Chesapeake Bay. Proc. N.E. Weed. Sci. Soc. 32:339 (Abstract).

Richard, J.J., G.A. Junk, M.J. Avery, N.L. Nehring, J.S. Fritz and H.J. Svec. 1975. Analysis of various Iowa waters for selected pesticides: Atrazine, DDE and Dieldrin-1974. Pesticides Monitor. J. 9:117-123.

Roberts, G.C., G.J. Sirons, R. Frank and H.E. Collins. 1979. Triazine residues in a watershed in southwestern Ontario (1973-1975). Jour. Great Lakes Research 5:246-255.

Schepers, J.S., D.R. Anderson, G.E. Schuman, E.J. Vavricka and H.D. Wittmuss. 1978. Agricultural runoff in the midwest. Nebraska Agric. Exp. Station Public No. 5643.

Stevenson, J.C. and N.M. Confer. 1978. Summary of available information on Chesapeake Bay submerged vegetation. U.S. Dept. Inter. IWS/OBS-78/66. NTIS, Springfield, VA. 333 pp.

United States Geological Survey. 1983. Estimated streamflow entering Chesapeake Bay. U.S.G.S., 208 Carroll Blvd., LaSalle Road, Towson, MD.

Waldron, A.C. and C.W. Bailey. 1974. Pesticide movement from cropland into Lake Erie. Environmental Protection Agency Technical Series. EPA-660/2-74-033, Washington, DC.

Walsh, G.E., D.L. Hansen and D.A. Lawrence. 1982. A flow-through system for exposure of seagrass to pollutants. Mar. Envir. Res. 7:1-11.

Walsh, J.J. 1976. Herbivory as a factor in patterns of nutrient utilization in the sea. Limnol. Oceanogr. 21:1-13.

Wauchope, R.D. and R.A. Leonard. 1980. Maximum pesticide concentrations in agricultural runoff: a semi-empirical formula. J. Environ. Qual. 9:665-672.

Wetzel, R.L., P.A. Penhale and K.L. Webb. 1981. Plant community structure and physical-chemical regimes at the Vaucluse Shores study site. Chap. 2, Sect. A. Virginia Inst. Mar. Sci., Gloucester Pt., VA.

Wu, T.L. 1980. Dissipation of the herbicides atrazine and alachlor in a Maryland corn field. J. Environ. Qual. 9:459-465.

Zahnow, E.W. and J.C. Riggleman. 1980. Search for linuron residues in tributaries of the Chesapeake Bay. J. Agric. Food Chem. 28:974-978.

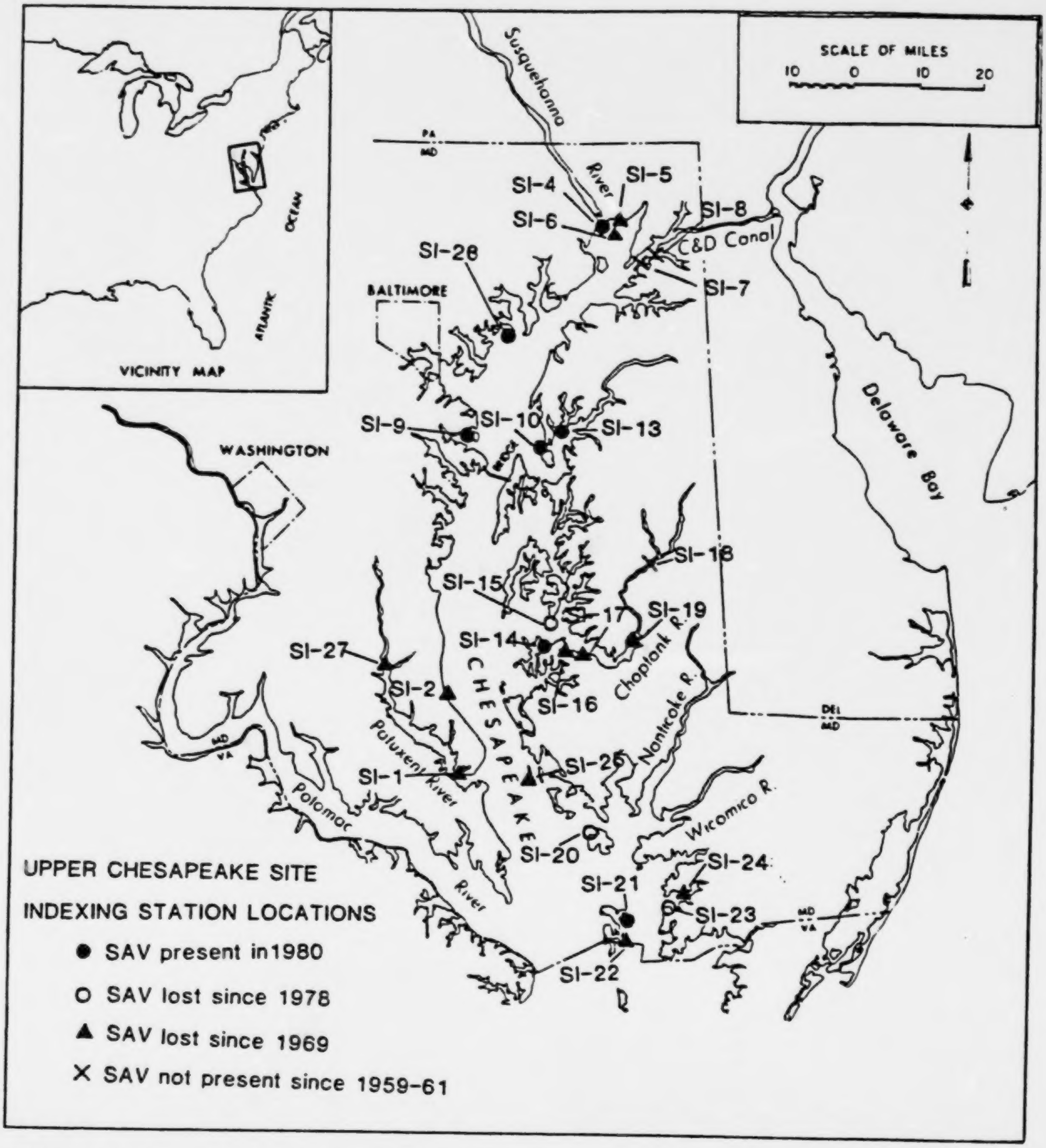
SCALE OF MILES
10 0 10 20
VICINITY MAP
ATLANTIC
OCEAN
Susquehanna River
PA
MD
SI-4
SI-5
SI-6
SI-8
C&D Canal
SI-7
SI-28
BALTIMORE
Delaware Bay
SI-9
SI-10
SI-13
WASHINGTON
SI-18
SI-15
SI-17
SI-19
Choptank R.
SI-14
SI-27
SI-2
CHESAPEAKE
SI-16
DEL
MD
Nanticoke R.
Patuxent River
SI-1
SI-25
MD
VA
Potomac River
Wicomico R.
SI-20
SI-24
SI-21
SI-23
SI-22
UPPER CHESAPEAKE SITE
INDEXING STATION LOCATIONS
● SAV present in 1980
○ SAV lost since 1978
▲ SAV lost since 1969
× SAV not present since 1959-61

Figure 1. Location and coding of sites investigated during the littoral zone survey conducted in upper Chesapeake Bay during 1980. Latitude, longitude and other station characteristics are given in Table 1.

MAP CODE	RIVER MILE	STATION NAME
1	0	River Mouth
2	3.5	Todds Point
3	7.6	Chlora Point
4	8.4	Lecompte Bay
5	10.6	Howell Point
6	10.8	Hambrook Bar
7	16.2	Marker "31"
8	17.7	Goose Pt.
9	19.4	Jamaica Point
10	21.0	Cabin Creek
11	22.4	Blinkhorn Creek
12	23.7	Marker "41"
13	26.8	Lloyds Landing
14	29.5	Frazer Neck
15	30.3	Marker "55"
16	33.3	Kingston Landing
17	35.9	Crowberry Creek
18	38.4	Marker "64"

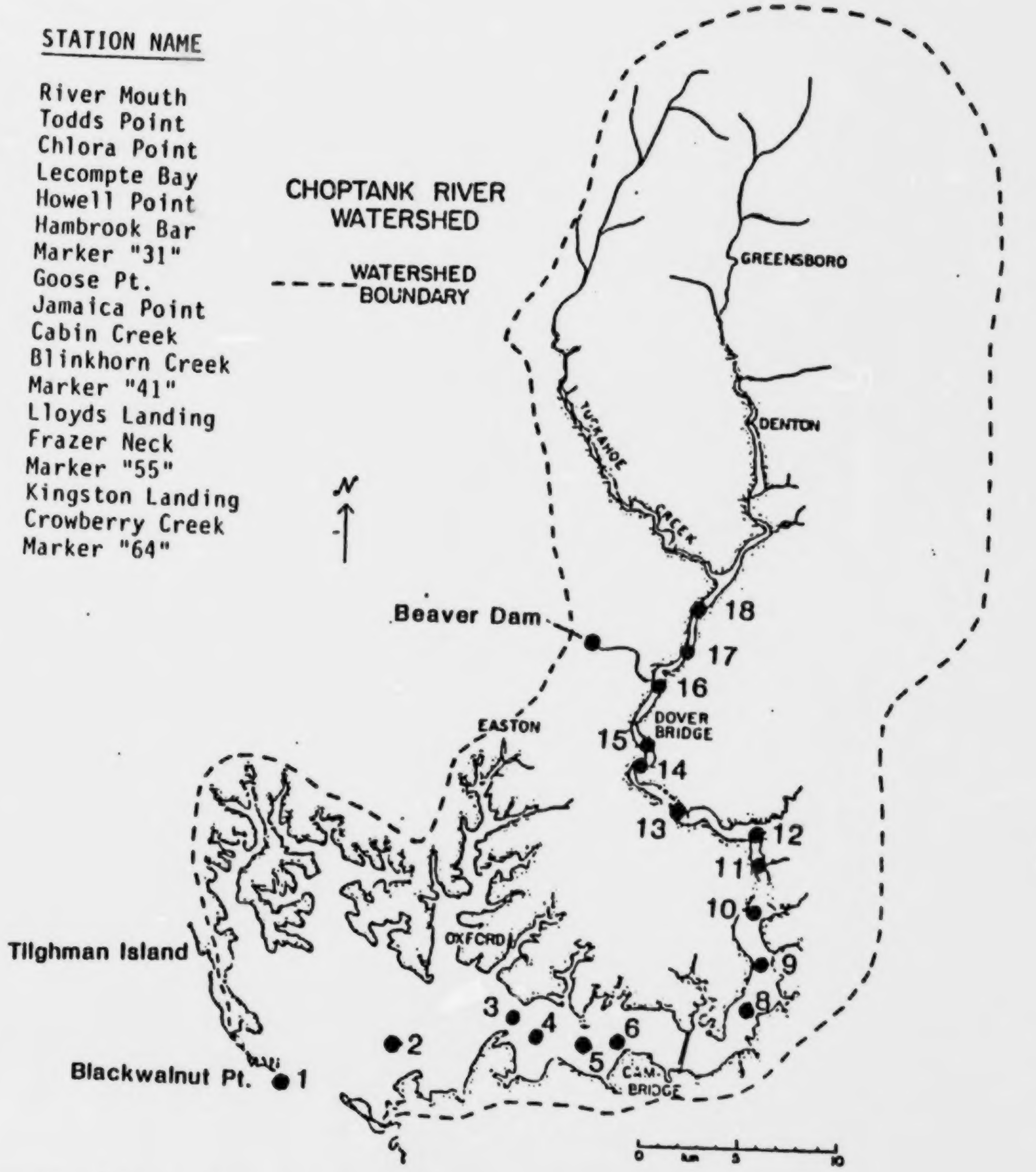
CHOPTANK RIVER WATERSHED
WATERSHED BOUNDARY
N
GREENSBORO
DENTON
TUCKAHOE CREEK
Beaver Dam
EASTON
DOVER BRIDGE
OXFORD
Tilghman Island
Blackwalnut Pt.
CAMBRIDGE
1
2
3
4
5
6
8
9
10
11
12
13
14
15
16
17
18
0
5
10

Figure 2. Location of stations in the Choptank River estuary where samples were taken and analyzed for dissolved and particulate herbicide (atrazine andlinuron) residues during 1980.

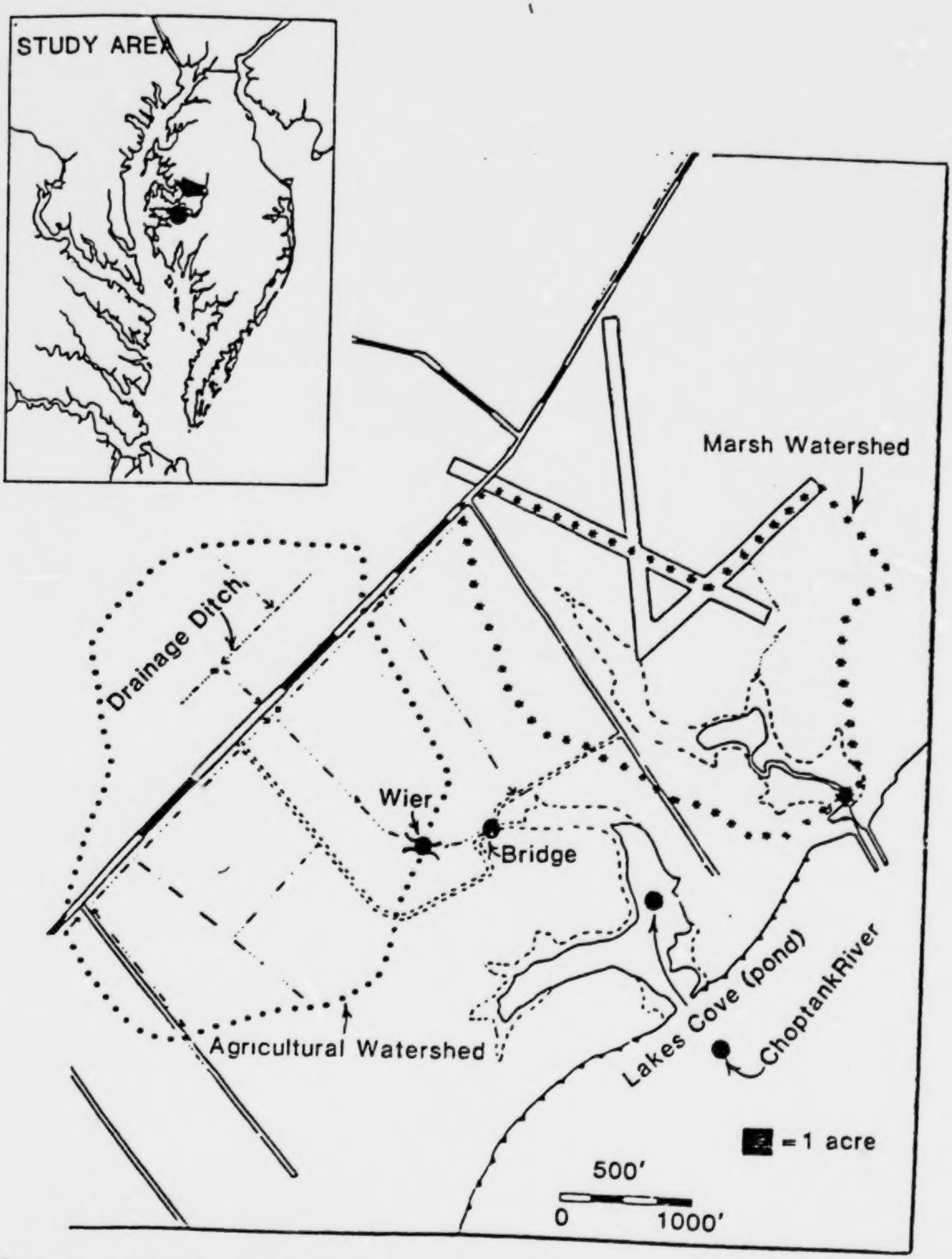
STUDY AREA
Marsh Watershed
Drainage Ditch
Wier
Bridge
Agricultural Watershed
Lakes Cove (pond)
ChoptankRiver
■ =1 acre
500'
0
1000'

Figure 3. Configuration and sampling station locations at the Horn Point experimental watershed located adjacent to the Choptank River estuary.

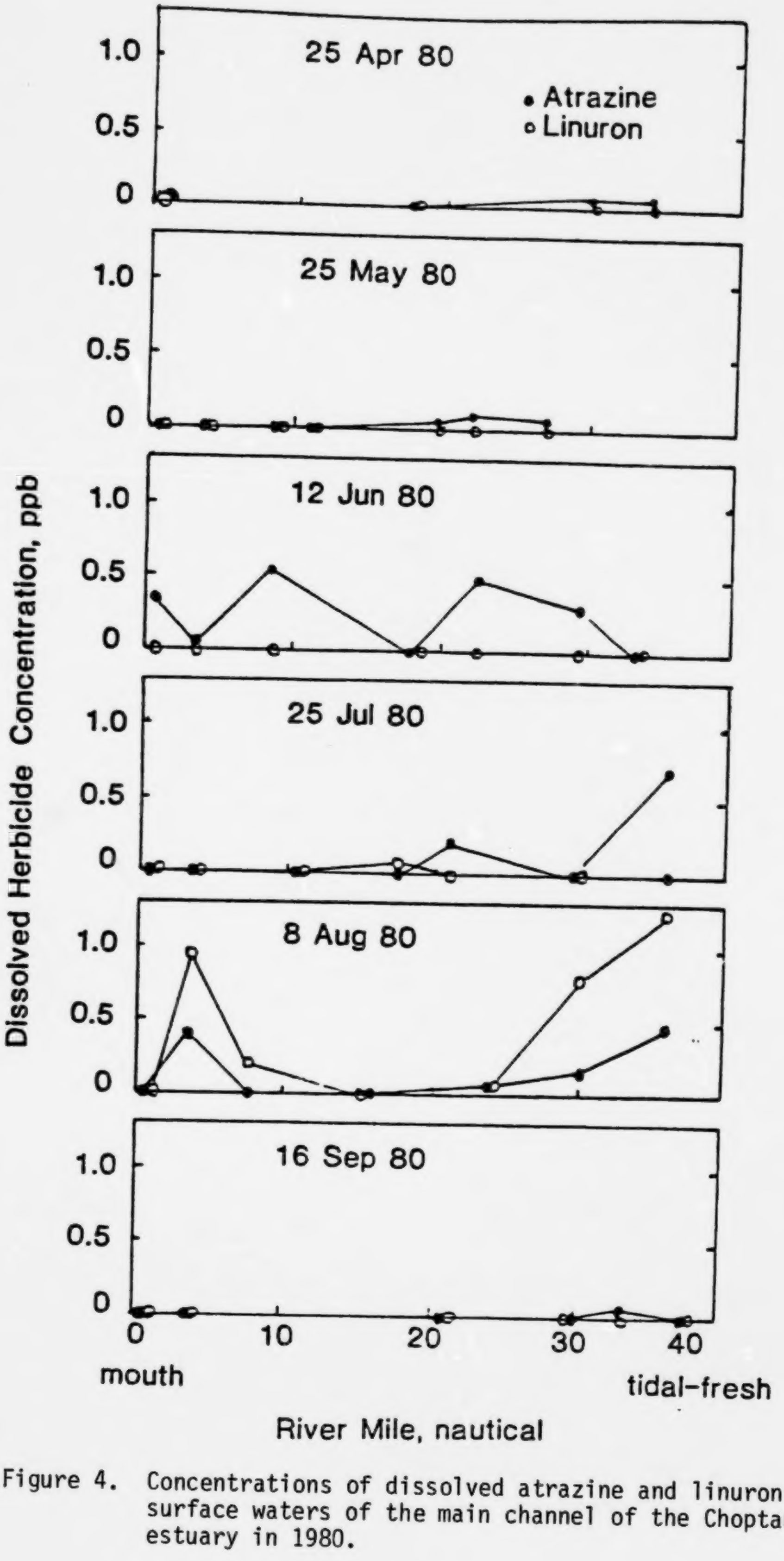

Figure 4. Concentrations of dissolved atrazine and linuron in surface waters of the main channel of the Choptank River estuary in 1980.

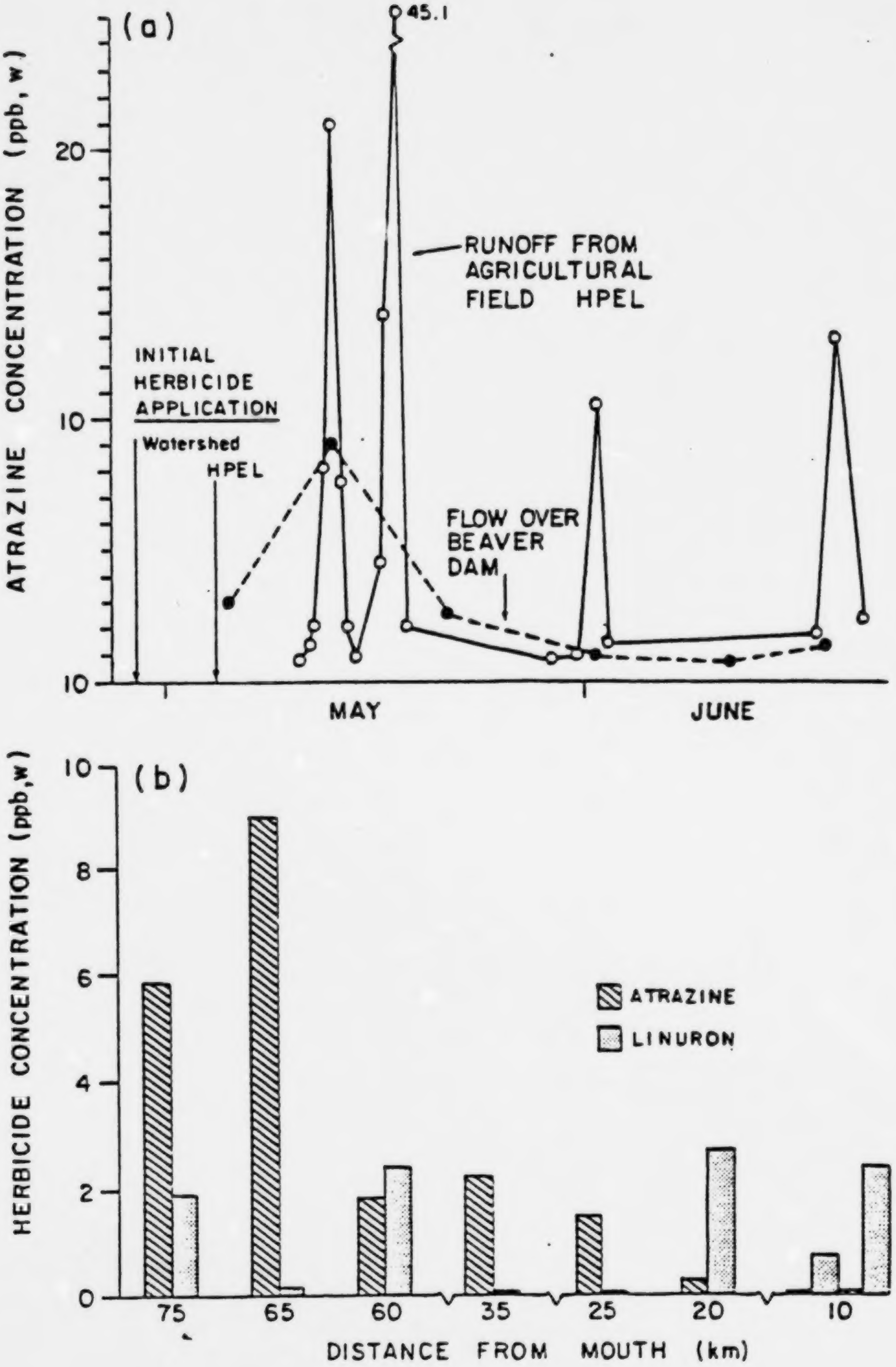

Figure 5. Temporal patterns of atrazine concentrationsin runoff from 2 agricultural watersheds in the Choptank River basin during the spring of 1981 (a) spatial distribution of herbicides along the longitudinal axis of the Choptank River estuary from May 10-13, 1981 (b) and details of rainfall at the Horn Point watershed, runoff from two sites and dissolved atrazine concentrations measured at the flume at HOrn Point and at Beaverdam (c). Data are presented in Appendix Table 9.

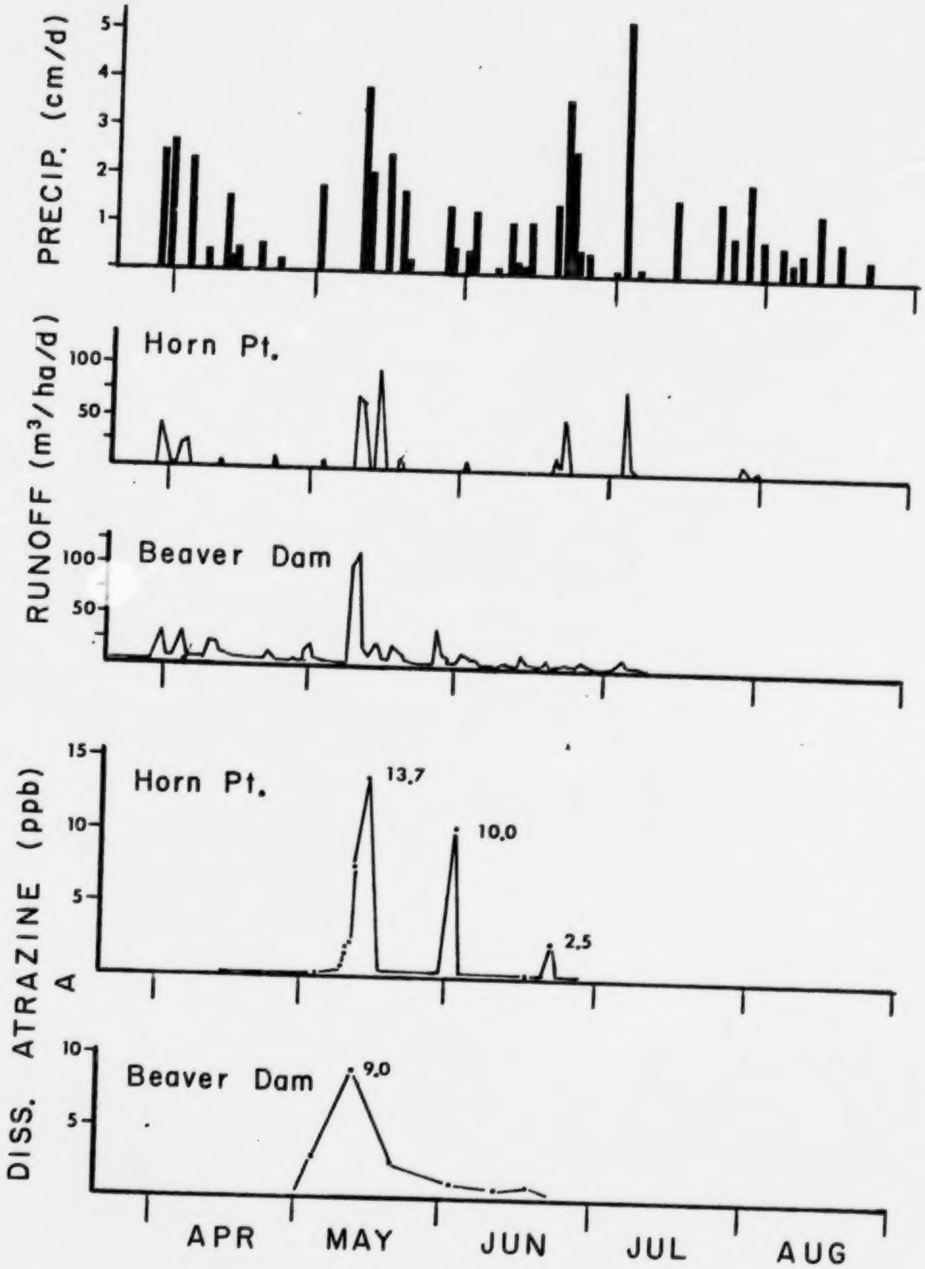
PRECIP. (cm/d)
5
4
3
2
1
RUNOFF (m³/ha/d)
Horn Pt.
100
50
Beaver Dam
100
50
DISS. ATRAZINE (ppb)
Horn Pt.
15
10
5
13.7
10.0
2.5
Beaver Dam
10
5
9.0
APR
MAY
JUN
JUL
AUG

Figure 5c.

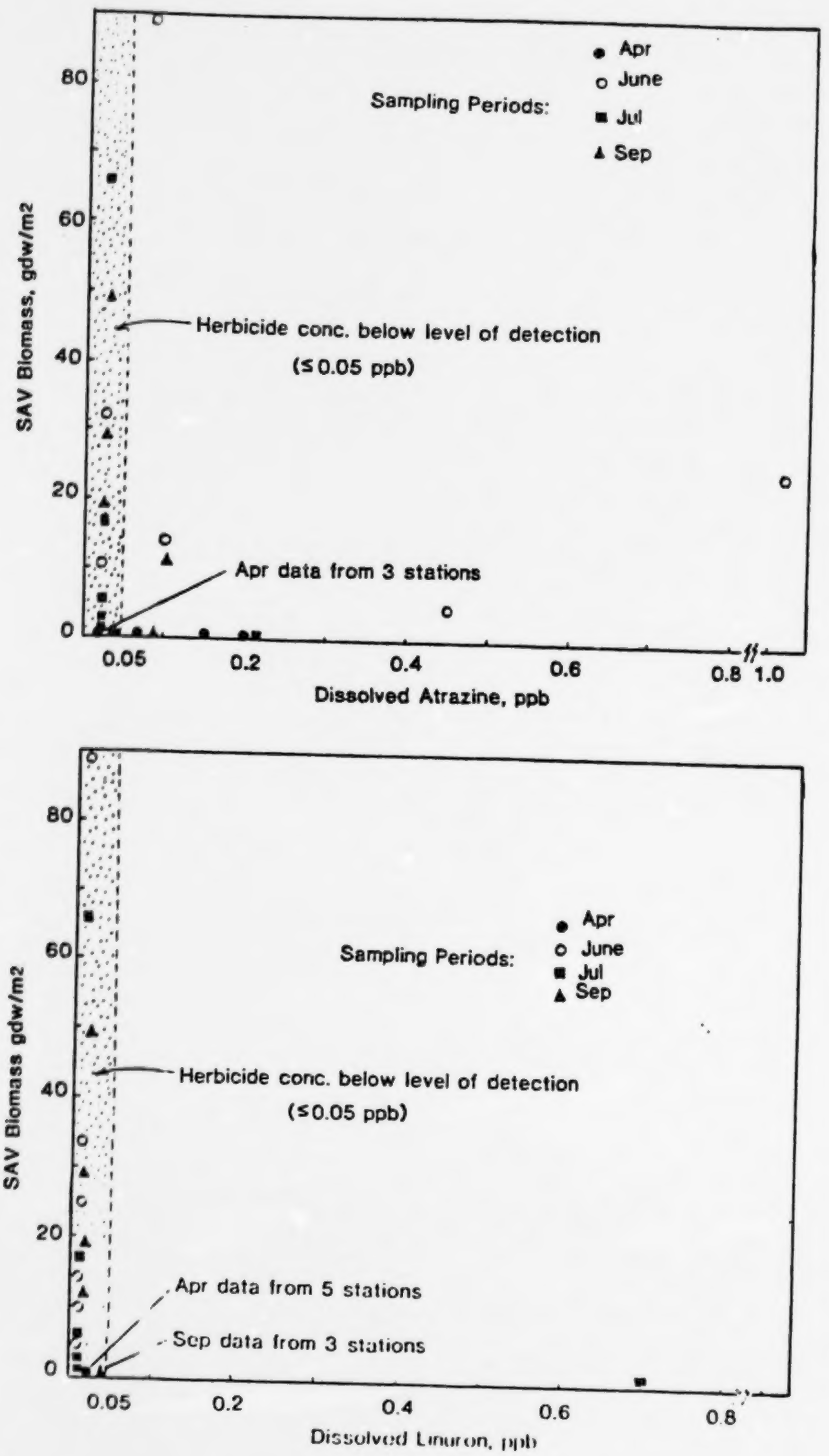

Figure 6. Scatter diagram of SAV biomass versus dissolved atrazine (a) and dissolved linuron (b) concentrations observed at littoral zone stations in upper Chesapeake Bay in 1980.

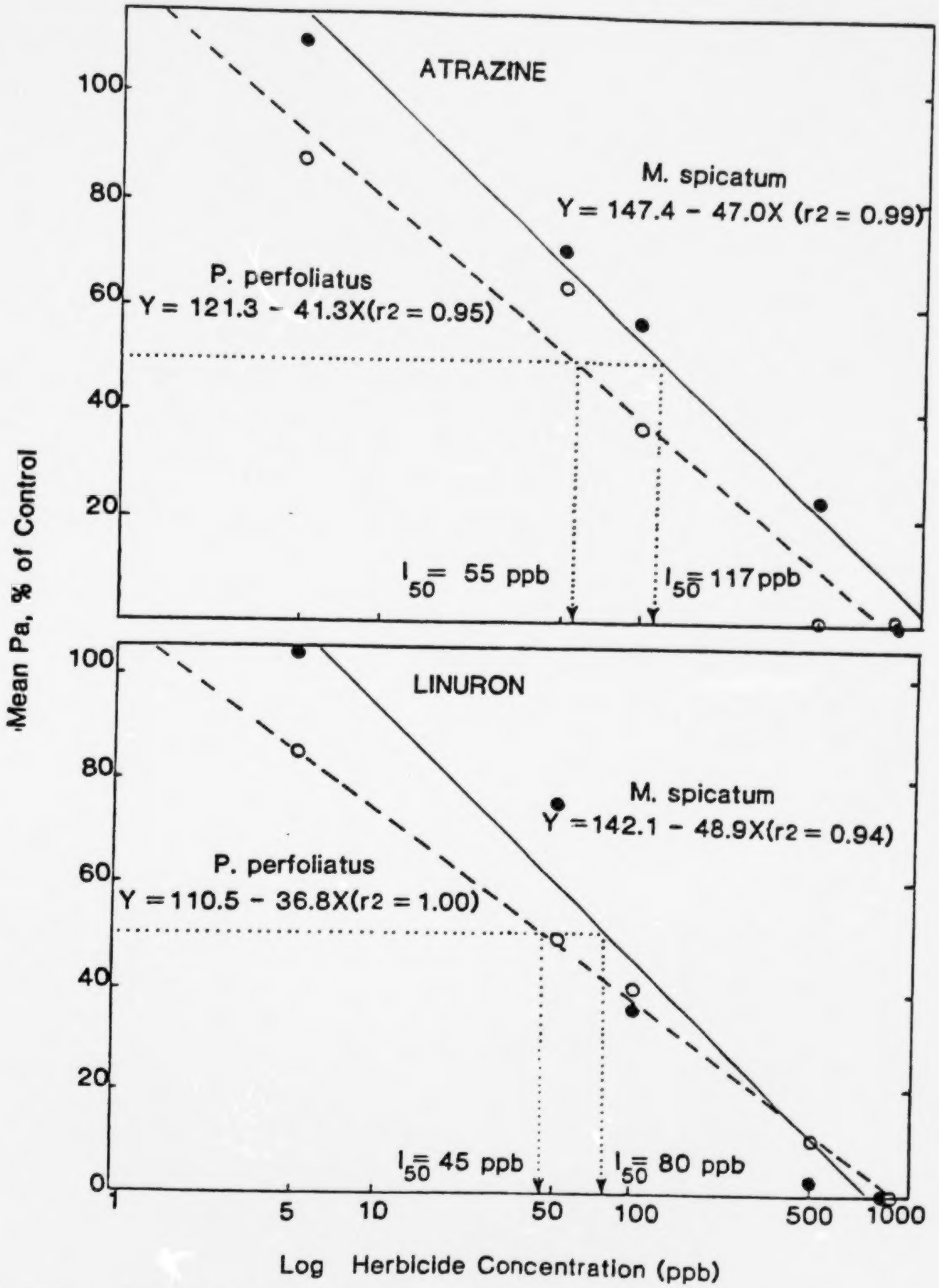

Figure 7. Regressions of mean apparent O_2 production (Pa; expressed at % of control value) versus the log of herbicide concentration for P. perfoliatus and M. spicatum treated with (a) atrazine and (b) linuron. Also shown are I_{50} values (herbicide concentration predicted to cause 50% photosynthetic inhibition) for each species. Diagram was adapted from Kemp et al. (1983).

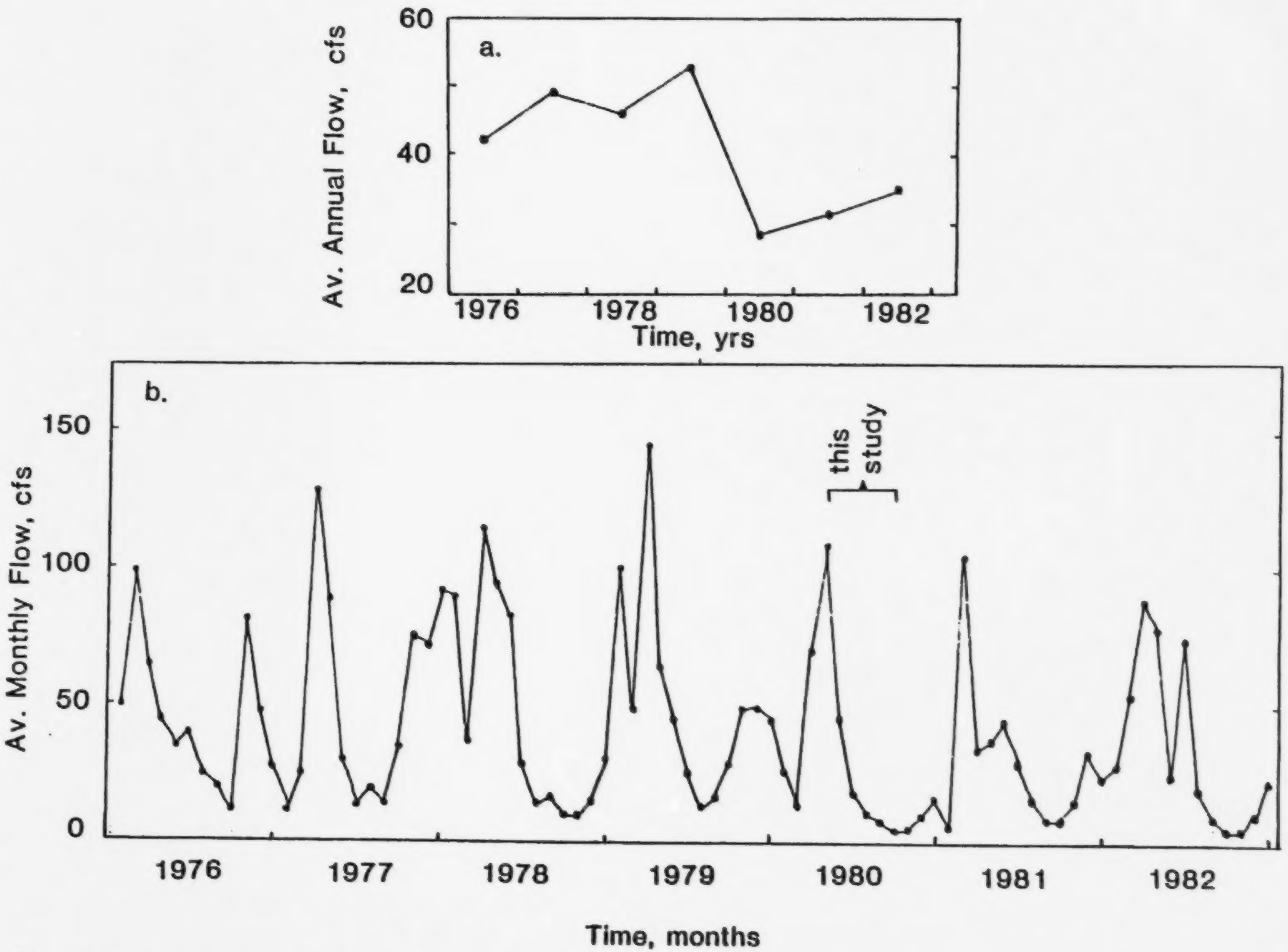

Figure 8. Freshwater discharge from the Susquehanna River (measured at Conowingo Dam) expressed at (a) average annual flow and (b) average monthly flow for the period 1976-1982. Data were compiled from U.S.G.S. (1983).

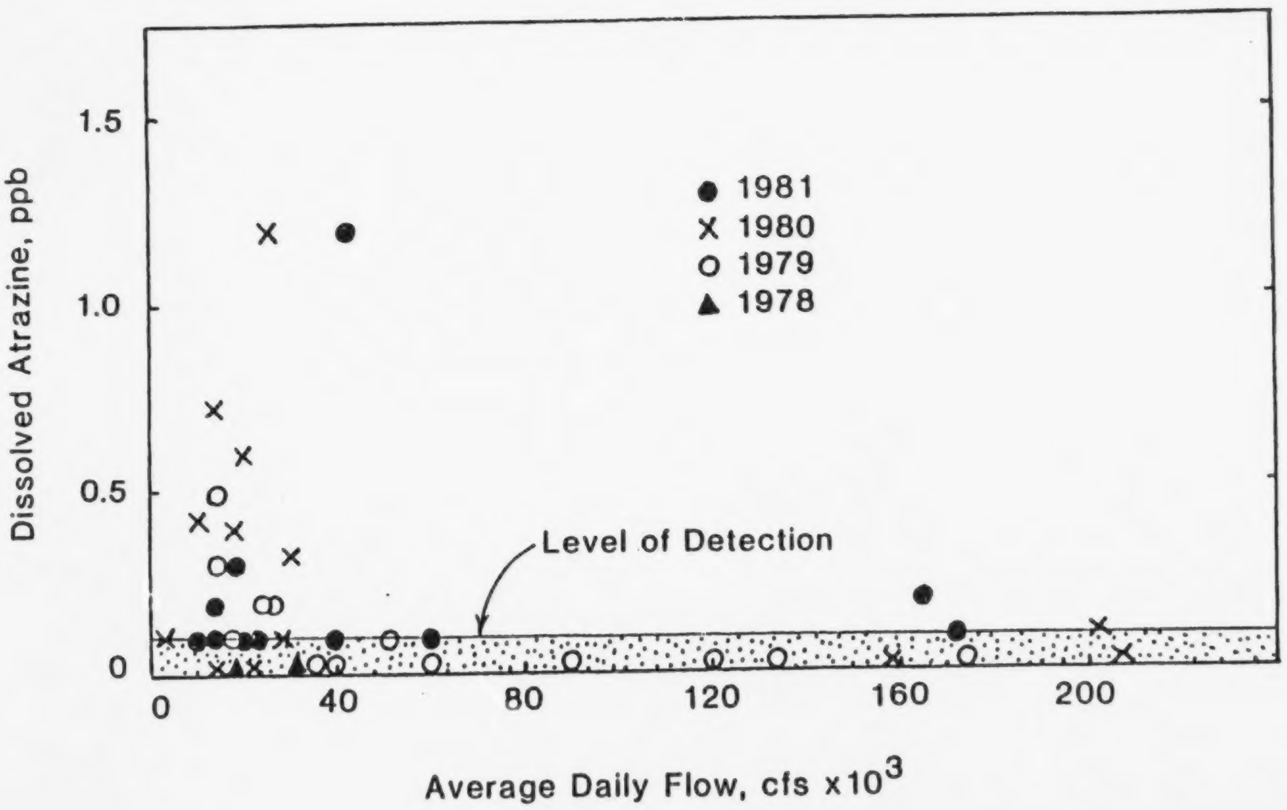

Figure 9. Scatter diagram of dissolved atrazine concentration in the Susquehanna River versus average daily flow of the Susquehanna measured at Conowingo Dam. Data were compiled from U.S.G.S. (1983).

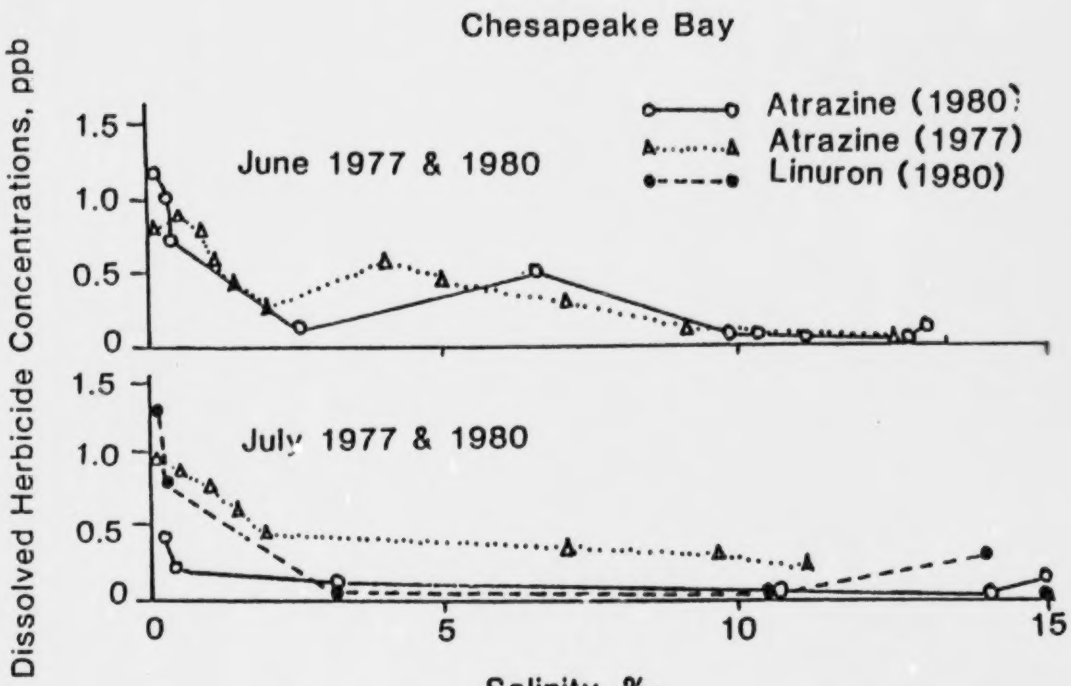

Figure 10. Concentrations of dissolved atrazine and linuron in surface waters of Chesapeake Bay for June and July of 1977 and 1980. Data for 1980 are from this study; data for 1977 are from Austin (see Stevenson and Confer, 1978) and Newby et al. (1978).

Table 1. Station coding, location and site descriptors associated with Upper Bay site indexing study, 1980. Units are: silt-clay content, %; median diameter, mm; light extinction, m^{-1} (k=extinction coefficient).

				SEDIMENT TYPE		
SAV STATUS	STATION CODE	LOCATION	LATITUDE/ LONGITUDE	PERCENT SILT/CLAY	MEDIAN DIAMETER	LIGHT EXTINCTION
I SAV Present 1980	SI-4	Susquehanna Mouth Concord Point	39°32'08" 76°05'17"	55.8±24.4	0.20±0.08	1.5±0.52
	SI-9	Magothy River James Pond	39°05'33" 76°26'07"	17.0±1.3	0.20±0.02	2.5±1.4
	SI-10	Chester R. Mouth E. Neck Narrows	39°02'54" 76°13'42"	9.0±4.7	0.13±0.01	1.4±0.3
	SI-13	Chester River Cliffs Bight	39°05'17" 76°09'30"	32.0±23.5	0.20±0.03	1.7±0.2
	SI-14	Choptank River Todds Cove	38°36'38" 76°14'00"	34.9±24.4	0.14±0.01	1.7±0.6
	SI-21	Smith Island Otter Is.	37°58'16" 75°59'42"	54.6±7.7	0.12±0.0	1.9±1.0
	SI-28	Gunpowder R. Mouth Lower Is. Pt.	39°18'34" 76°20'40"	45.2±11.0	0.20±0.03	1.1±0.6
II SAV Lost Since 1978	SI-15	Choptank River Benoni Pt.	38°41'03" 76°12'54"	25.1±14.3	0.13±0.0	1.3±0.5
	SI-20	South Marsh Is. Johnson Pt.	38°06'37" 76°03'18"	63.5±24.5	0.16±0.02	2.3±0.8
	SI-23	Big Annemessex R. Wear Point	38°02'33" 75°49'49"	42.4±14.0	0.37±0.06	1.7±0.9
III SAV Lost Since 1969	SI-1	Patuxent R. Mouth Solomons Is.	38°19'07" 76°27'09"	0.9±0.5	0.25±0.09	1.5±0.9
	SI-2	Mid-Chesapeake Western Shores	38°29'26" 76°29'45"	0.8±0.7	.20±.01	1.8±0.6
	SI-5	Susquehanna Mouth Furnace Bay	39°33'17" 76°02'38"	60.1±33.4	.17±.03	2.3±0.6
	SI-6	Susquehanna Flats	39°30'42" 76°04'16"	1.4±1.9	.23±.02	1.8±0.7
	SI-16	Choptank River Lecompte Cr.	38°36'11" 76°10'35"	31.8±10.9	.19±.01	2.0±1.7
	SI-17	Choptank River Horn Point	38°35'38" 76°07'52"	52.0±0.0	1.0±.00	1.0±0.0
	SI-19	Choptank River Frazier Neck	38°44'00" 76°00'09"	2.7±0.5	.38±.05	4.4±2.7
	SI-22	Smith Island Horse Hammoch	37°56'57" 75°59'42"	80.1±8.2	.14±.02	3.9±1.5
	SI-24	Big Annemessex R. Moon Bay	38°04'12" 75°47'42"	29.3±24.3	.39±.03	2.4±0.8
	SI-25	Barren Island Sand Pt.	38°18'45" 76°15'00"	52.4±30.3	.16±.03	2.6±1.8
	SI-27	Patuxent River Buena Vista	38°30'46" 76°39'47"	0.6±0.2	2.0±.00	2.9±0.2
IV SAV Not Present (at least) since 1959-61)	SI-7	Elk River Cabin John Cr.	39°27'34" 75°57'11"	28.8±4.2	.14±.01	4.0±0.9
	SI-8	Elk River Veazey Neck	39°28'11" 75°57'01"	2.7±2.8	.72±.86	2.6±0.4
	SI-18	Choptank River Kingston Ldg.	38°46'26" 75°57'57"	51.8±12.9	.29±.15	4.1±1.3

Table 2. Summary of maximum and average dissolved atrazine (A) and linuron (B) concentrations measured in various geographical areas of the upper Chesapeake Bay during 4 sampling periods in 1980.

	Sampling Periods			
	April	June	July	September
A. Atrazine				
• Maximum Observed Concentration	0.3	1.1-1.2	0.4	0.9
• Percent Observations				
≥0.5 ppb	0	27	0	9
≥0.1 ppb	36	32	35	17
• Mean Concentrations by Geographical Area[a]				
Upper Bay	0.09	0.78	0.18	≤0.05
Upper-Mid Bay	≤0.05	0.18	≤0.05	≤0.05
Mid Bay	0.11	0.13	≤0.05	0.16
Lower Bay	0.11	≤0.05	0.07	≤0.05
Tidal Fresh Tribs.	≤0.05	≤0.05	0.12	0.30
Stations with SAV	0.08	0.29	≤0.05	≤0.05

	Sampling Periods			
	April	June	July	September
B. Linuron				
• Maximum Observed Concentration	≤0.05	≤0.05	1.3	0.75
• Percent Observations				
≥0.50 ppb	0	0	15	9
≥0.10 ppb	0	0	20	13
• Mean Concentrations by Geographical Area[a]				
Upper Bay	≤0.05	≤0.05	0.42	0.12
Upper-Mid Bay	≤0.05	≤0.05	≤0.05	≤0.05
Mid Bay	≤0.05	≤0.05	0.05	≤0.05
Lower Bay	≤0.05	≤0.05	≤0.05	≤0.05
Tidal Fresh Tribs.	≤0.05	≤0.05	0.20	0.25
Stations with SAV	≤0.05	≤0.05	0.13	≤0.05

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[a] Stations were grouped according to geographical areas as follows:
Upper Bay: SI's 4, 5, 6, 7 and 8.
Mid-Upper Bay: SI's 9, 10, 13 and 28.
Mid Bay: SI's 1, 2, 14, 15, 16 and 15.
Lower Bay: SI's 20, 21, 22, 23 and 24.
Tidal Fresh Tributaries: SI's 18, 19 and 27.
Stations with SAV: SI's 4, 9, 10, 13, 14, 21 and 28; Refer to Fig. 1 for locations.

Table 3. Station locations at which maximum dissolved atrazine (A) and linuron (B) concentrations (ppb) were observed during 1980 littoral zone surveys.

A. Atrazine

Locations	Sampling Periods April	 June	 July	 September
Upper Bay	0.20(SI-7)	1.14(SI-6)[b]	0.4(SI-8)	0.11(SI-8
Upper-Mid Bay	≤0.05	0.45(SI-13)	≤0.05	0.10(SI-13)
Mid Bay	0.30(SI-1)	0.65(SI-2)	0.13(SI-1)	0.93(SI-1
Lower Bay	0.30(SI-24)	0.10(SI-21)	0.20(SI-22)	≤0.05
Tidal Fresh Tribs.	0.10(SI-18)	0.10(SI-19)	0.25 (SI-27)	0.91(SI-27)
Stations with SAV	0.20(SI-21)	1.09(SI-4)	0.20(SI-4)	0.10(SI-13)

B. Linuron

Locations[a]	Sampling Periods April	 June	 July	 September
Upper Bay	≤0.05	≤0.05	1.30(SI-7)[c]	0.56(SI-8)
Upper-Mid Bay	≤0.05	≤0.05	≤0.05	≤0.05
Mid Bay	≤0.05	≤0.05	0.25(SI-1)	0.31(SI-1)
Lower Bay	≤0.05	≤0.05	≤0.05	≤0.05
Tidal Fresh Tribs.	≤0.05	≤0.05	0.55(SI-27)	0.75(SI-27)
Stations with SAV	≤0.05	≤0.05	0.80(SI-4)	≤0.05

[a] See Table 2 for station groupings and Fig.1 for locations.

[b] At other upper bay stations atrazine concentrations (ppb) were 1.09(SI-4), ≤0.05(SI-5), 1.08(SI-7) and 0.7(SI-8).

[c] Linuron concentration was 0.8 ppb at Station SI-4 (upper bay area).

Table 4. Stations and dates at which detectable (≥0.1 μg kg^{-1}) concentrations of atrazine and linuron were found in surficial sediments collected from littoral zone sites in upper Chesapeake Bay, 1980.

		Herbicide Concentration, μg kg^{-1}	
Date	Station	Atrazine	Linuron
April 28	SI-23	0.37	≤0.10
June 13	SI-5	0.65	≤0.10
June 13	SI-7	0.43	≤0.10
June 12	SI-18	0.64	≤0.10
Sept. 17	SI-28	0.93	≤0.10
Sept. 15	SI-23	≤0.10	0.51

Table 5. Summary of mean SAV aboveground biomass (gdw/m^2) measured on 4 occasions in upper Chesapeake Bay, 1980.

DATE	UPPER BAY		MID-BAY				LOWER BAY
	SI-4	SI-28	SI-9	SI-10	SI-13	SI-14	SI-21
April	0	0	0	0	0	0	0
June	24.8	91.2	---	10.3	4.6	33.2	14.4
July	0.0	17.2	0.8	2.0	5.6	66.0	35.6
Sept	0.0	0.0	0.0	19.2	12.0	49.2	29.2

Appendix Table 1. Listing of dissolved herbicide concentrations (ppb), SAV biomass and other selected variables measured at 23 littoral zone stations in upper Chesapeake Bay, 1980. See text for explanation of SAV status. Temperature in ^{o}C, salinity as ppt, and SAV biomass as gdw/m^{2}. All water quality samples were taken 0.5m below the surface.

SAV STATUS	STATIONS	DATE	TEMP	SALINITY	HERBICIDE CONC (ppb)		SAV	
		April 1980 Cruise			Atrazine	Linuron	Biomass	Species
I	SI-4	24/4	13.2	0.0	0.07	≤0.05	0.0	-
	SI-9	24/4	18.7	3.3	≤0.05	≤0.05	0.0	-
	SI-10	24/4	19.2	5.2	≤0.05	≤0.05	0.0	-
	SI-13	25/4	16.2	5.5	≤0.05	≤0.05	0.0	-
	SI-14	25/4	17.8	9.6	0.15	≤0.05	0.0	-
	SI-21	28/4	16.3	11.7	0.20	≤0.05	0.0	-
	SI-28	NOT SAMPLED						
II	SI-15	25/4	18.8	9.8	0.10	≤0.05	0.0	-
	SI-20	28/4	15.9	11.9	≤0.05	≤0.05	0.0	-
	SI-23	28/4	17.0	11.1	≤0.05	≤0.05	0.0	-
III	SI-1	23/4	15.6	8.7	0.30	≤0.05	0.0	-
	SI-2	23/4	17.8	7.6	≤0.05	≤0.05	0.0	-
	SI-5	24/4	13.6	0.0	0.15	≤0.05	0.0	-
	SI-6	24/4	13.6	0.0	≤0.05	≤0.05	0.0	-
	SI-16	25/4	19.3	9.1	≤0.05	≤0.05	0.0	-
	SI-19	25/4	18.0	0.9	≤0.05	≤0.05	0.0	-
	SI-22	28/4	15.8	11.6	≤0.05	≤0.05	0.0	-
	SI-24	28/4	17.5	10.9	0.30	≤0.05	0.0	-
	SI-25	28/4	15.3	8.7	0.10	≤0.05	0.0	-
	SI-27	1/5	15.4	3.2	≤0.05	≤0.05	0.0	-
IV	SI-7	25/4	17.3	0.0	0.20	≤0.05	0.0	-
	SI-8	24/4	16.8	0.0	≤0.05	≤0.05	0.0	-
	SI-18	25/4	18.0	0.4	0.10	≤0.05	0.0	-

Appendix Table 1 (cont.)

SAV STATUS	STATIONS	DATE	TEMP	SALINITY	HERBICIDE CONC (ppb)		SAV	
		June 1980 Cruise			Atrazine	Linuron	Biomass	Species
I	SI-4	13/6	20.5	0.1	1.09	≤0.05	24.8	M. spicatum
	SI-9	NOT SAMPLED						
	SI-10	12/6	24.8	9.3	≤0.05	≤0.05	10.3	mixed[a]
	SI-13	12/6	25.5	6.5	0.45	≤0.05	4.6	R. maritina P. perfoliatus
	SI-14	12/6	23.8	10.0	≤0.05	≤0.05	33.2	P. perfoliatus
	SI-21	11/6	22.4	12.8	0.10	≤0.05	14.4	Z. marina
	SI-28	13/6	24.0	2.3	0.09	≤0.05	91.2	P. perfoliatus
II	SI-15	12/6	24.9	9.9	≤0.05	≤0.05	0.0	-
	SI-20	11/6	24.5	12.2	≤0.05	≤0.05	0.0	-
	SI-23	11/6	21.1	12.9	≤0.05	≤0.05	0.0	-
III	SI-1	16/6	23.4	13.0	≤0.05	≤0.05	0.0	-
	SI-2	16/6	21.9	12.3	0.65	≤0.05	0.0	-
	SI-5	15/6	19.5	0.1	≤0.05	≤0.05	0.0	-
	SI-6	13/6	22.5	0.1	1.14	≤0.05	0.0	-
	SI-16	12/6	21.8	9.5	≤0.05	≤0.05	0.0	-
	SI-19	12/6	-	-	0.10	≤0.05	0.0	-
	SI-22	11/6	21.5	13.0	≤0.05	≤0.05	0.0	-
	SI-24	11/6	20.7	13.2	≤0.05	≤0.05	0.0	-
	SI-25	11/6	22.4	11.2	≤0.05	≤0.05	0.0	-
	SI-27	16/6	24.7	7.7	≤0.05	≤0.05	0.0	-
IV	SI-7	13/6	22.0	0.3	1.08	≤0.05	0.0	-
	SI-8	13/6	22.0	0.2	0.70	≤0.05	0.0	-
	SI-18	12/6	21.1	0.6	≤0.05	≤0.05	0.0	-

Appendix Table 1 (cont.)

SAV STATUS	STATIONS	DATE	TEMP	SALINITY	HERBICIDE CONC (ppb)		SAV	
		July 1980 Cruise			Atrazine	Linuron	Biomass	Species
I	SI-4	26/7	27.3	0.2	0.20	0.80	0.0	-
	SI-9	26/7	31.9	8.4	≤0.05	≤0.05	0.8	*V. americana*
	SI-10	25/7	-	-	≤0.05	≤0.05	2.0	*P. perfoliatus*
	SI-13	25/7	31.8	7.3	≤0.05	≤0.05	5.6	*P. perfoliatus* *R. maritima*
	SI-14	24/7	28.6	9.8	≤0.05	≤0.05	66.0	*P. perfoliatus*
	SI-21	24/7	26.7	14.7	NOT SAMPLED		35.6	*Z. marina*
	SI-28	25/7	26.7	3.8	≤0.05	≤0.05	17.2	*P. perfoliatus*
II	SI-15	24/7	29.6	10.5	≤0.05	≤0.05	0.0	-
	SI-20	24/7	28.9	12.8	NOT SAMPLED		0.0	-
	SI-23	24/7	25.7	14.1	NOT SAMPLED		0.0	-
III	SI-1	29/7	28.3	14.0	0.13	0.25	0.0	-
	SI-2	26/7	28.5	12.6	≤0.05	≤0.05	0.0	-
	SI-5	26/7	26.2	0.3	≤0.05	≤0.05	0.0	-
	SI-6	26/7	30.1	0.1	0.20	≤0.05	0.0	-
	SI-16	24/7	28.4	10.4	≤0.05	≤0.05	0.0	-
	SI-19	25/7	28.7	3.1	0.10	≤0.05	0.0	-
	SI-22	24/7	26.2	15.2	0.20	≤0.05	0.0	-
	SI-24	24/7	25.5	13.7	≤0.05	≤0.05	0.0	-
	SI-25	24/7	29.0	12.9	≤0.05	≤0.05	0.0	-
	SI-27	29/7	28.2	10.0	0.25	0.55	0.0	-
IV	SI-7	26/7	31.2	0.2	0.20	1.30	0.0	-
	SI-8	26/7	29.7	0.2	0.40	≤0.05	0.0	-
	SI-18	25/7	28.4	1.7	≤0.05	≤0.05	0.0	-

Appendix Table 1 (cont.)

SAV STATUS	STATIONS	DATE	TEMP	SALINITY	HERBICIDE CONC (ppb)		SAV	
	September 1980 Cruise				Atrazine	Linuron	Biomass	Species
I	SI-4	18/9	20.5	0.2	≤0.05	≤0.05	0.0	-
	SI-9	17/9	25.2	12.4	≤0.05	≤0.05	0.0	-
	SI-10	17/9	23.4	12.5	≤0.05	≤0.05	19.2	mixed[a]
	SI-13	17/9	22.2	10.9	0.10	≤0.05	12.0	*P. perfoliatus* *P. maritima*
	SI-14	16/9	23.1	15.2	≤0.05	≤0.05	49.2	*P. perfoliatus*
	SI-21	15/9	26.2	20.9	≤0.05	≤0.05	29.2	*Z. marina*
	SI-28	17/9	25.2	7.9	0.07	≤0.05	0.0	-
II	SI-15	16/9	23.8	14.9	≤0.05	≤0.05	0.0	-
	SI-20	15/9	24.9	18.8	≤0.05	≤0.05	0.0	-
	SI-23	15/9	25.8	19.6	≤0.05	≤0.05	0.0	-
III	SI-1	22/9	26.7	19.0	0.93	0.31	0.0	-
	SI-2	18/9	25.9	17.0	≤0.05	≤0.05	0.0	-
	SI-5	12/9	20.0	0.2	≤0.05	≤0.05	0.0	-
	SI-6	17/9	23.2	0.2	≤0.05	≤0.05	0.0	-
	SI-16	16/9	26.5	14.0	≤0.05	≤0.05	0.0	-
	SI-19	16/9	25.7	5.5	≤0.05	≤0.05	0.0	-
	SI-22	15/9	25.9	20.9	≤0.05	≤0.05	0.0	-
	SI-24	15/9	25.6	19.0	≤0.05	≤0.05	0.0	-
	SI-25	17/9	21.5	17.4	≤0.05	≤0.05	0.0	-
	SI-27	22/9	26.0	13.1	0.91	0.75	0.0	-
IV	SI-7	17/9	25.8	2.4	0.06	≤0.05	0.0	-
	SI-8	17/9	26.0	3.1	0.11	0.56	0.0	-
	SI-18	16/9	25.7	3.5	≤0.05	≤0.05	0.0	-

[a] Samples contained a mixture of the following species: *M. spicatuma*, *P. perfoliatus*, *E. canadensis* and *V. americana*.

Appendix Table 2. Listing of atrazine and linuron concentrations (ppb) in surficial sediment samples (upper 5 cm) collected from 23 littoral zone locations in upper Chesapeake Bay, 1980.

April 1980 Cruise		Sediment Herbicide Concentration (ppb)	
Station	**Date**	**Atrazine**	**Linuron**
SI-4	24/4	≤0.1	≤0.1
SI-9	24/4	≤0.1	≤0.1
SI-10	24/4	≤0.1	≤0.1
SI-13	24/4	≤0.1	≤0.1
SI-14	25/4	≤0.1	≤0.1
SI-21	NOT ANALYZED		
SI-28	NOT SAMPLED		
SI-15	NOT ANALYZED		
SI-20	28/4	≤0.1	≤0.1
SI-23	28/4	0.37	≤0.1
SI-1	23/4	≤0.1	≤0.1
SI-2	23/4	≤0.1	≤0.1
SI-5	24/4	≤0.1	≤0.1
SI-6	NOT ANALYZED		
SI-16	25/4	≤0.1	≤0.1
SI-19	25/4	≤0.1	≤0.1
SI-22	NOT ANALYZED		
SI-24	NOT ANALYZED		
SI-25	28/4	≤0.10	≤0.10
SI-27	1/5	≤0.10	≤0.10
SI-7	NOT ANALYZED		
SI-8	NOT ANALYZED		
SI-18	25/4	≤0.10	≤0.10

June 1980 Cruise

Station	Date	Sediment Herbicide Concentration (ppb) Atrazine	Linuron
SI-4	13/6	≤0.1	≤0.1
SI-9	NOT SAMPLED		
SI-10	12/6	≤0.1	≤0.1
SI-13	12/6	≤0.1	≤0.1
SI-14	12/6	≤0.1	≤0.1
SI-21	11/6	≤0.1	≤0.1
SI-28	13/6	≤0.1	≤0.1
SI-15	12/6	≤0.1	≤0.1
SI-20	11/6	≤0.1	≤0.1
SI-23	11/6	≤0.1	≤0.1
SI-1	16/6	≤0.1	≤0.1
SI-2	16/6	≤0.1	≤0.1
SI-5	13/6	0.65	≤0.1
SI-6	13/6	≤0.1	≤0.1
SI-16	12/6	≤0.1	≤0.1
SI-19	12/6	≤0.1	≤0.1
SI-22	11/6	≤0.1	≤0.1
SI-24	11/6	≤0.1	≤0.1
SI-25	11/6	≤0.1	≤0.1
SI-27	16/6	≤0.1	≤0.1
SI-7	13/6	0.43	≤0.1
SI-8	18/6	≤0.1	≤0.1
SI-18	12/6	0.64	≤0.1

July 1980 Cruise		Sediment Herbicide Concentration (ppb)	
Station	Date	Atrazine	Linuron
SI-4	26/7	≤0.1	≤0.1
SI-9	26/7	≤0.1	≤0.1
SI-10	25/7	≤0.1	≤0.1
SI-13	25/7	≤0.1	≤0.1
SI-14	24/7	≤0.1	≤0.1
SI-21	24/7	≤0.1	≤0.1
SI-28	25/7	≤0.1	≤0.1
SI-15	24/7	≤0.1	≤0.1
SI-20	24/7	≤0.1	≤0.1
SI-23	24/7	≤0.1	≤0.1
SI-1	29/7	≤0.1	≤0.1
SI-2	26/7	≤0.1	≤0.1
SI-5	26/7	≤0.1	≤0.1
SI-6	26/7	≤0.1	≤0.1
SI-16	24/7	≤0.1	≤0.1
SI-19	25/7	≤0.1	≤0.1
SI-22	23/7	≤0.1	≤0.1
SI-24	24/7	≤0.1	≤0.1
SI-25	24/7	≤0.1	≤0.1
SI-27	29/7	≤0.1	≤0.1
SI-7	NOT ANALYZED		
SI-8	26/7	≤0.1	≤0.1
SI-18	26/7	≤0.1	≤0.1

September 1980 Cruise

Station	Date	Sediment Herbicide Concentration (ppb) Atrazine	 Linuron
SI-4	17/9	≤0.1	≤0.1
SI-9	17/9	≤0.1	≤0.1
SI-10	17/9	≤0.1	≤0.1
SI-13	17/9	≤0.1	≤0.1
SI-14	16/9	≤0.1	≤0.1
SI-21	15/9	≤0.1	≤0.1
SI-28	17/9	0.93	≤0.1
SI-15	16/9	≤0.1	≤0.1
SI-20	15/9	≤0.1	≤0.1
SI-23	15/9	≤0.1	0.51
SI-1	22/9	≤0.1	≤0.1
SI-2	18/9	≤0.1	≤0.1
SI-5	18/9	≤0.1	≤0.1
SI-6	17/9	≤0.1	≤0.1
SI-16	16/9	≤0.1	≤0.1
SI-19	16/9	≤0.1	≤0.1
SI-22	15/9	≤0.1	≤0.1
SI-24	15/9	≤0.1	≤0.1
SI-25	16/9	≤0.1	≤0.1
SI-27	22/9	≤0.1	≤0.1
SI-7	17/9	≤0.1	≤0.1
SI-8	17/9	≤0.1	≤0.1
SI-18	16/9	≤0.1	≤0.1

Appendix Table 3. Summary of station locations, physical characteristics and dissolved herbicide concentrations (ppb) measured during monthly water quality surveys in the Choptank River Estuary, 1980. All samples were taken 0.5m from the surface. Station locations are shown in Fig. 2.

DATE	LOCATION	RIVER[a] MILE	SALINITY (o/oo)	TEMP (°C)	HERBICIDE CONC. Atrazine (ppb)	HERBICIDE CONC. Linuron (ppb)
25/4/80	Kingston Ldg.	33.3	0.5	18.0	0.08	≤0.05
	Frazer Neck	29.5	1.7	17.6	0.07	≤0.05
	Goost Pt.	17.7	6.7	17.1	≤0.05	≤0.05
	Choptank Mouth	0.0	8.9	14.5	0.06	≤0.05
27/5/80	Lloyds Ldg.	26.8	1.2	22.8	0.06	≤0.05
	Blinkhorn Cr.	22.4	3.1	22.2	0.10	≤0.05
	Jamaica Pt.	19.4	5.9	21.7	0.06	≤0.05
	Hambrook Bar	10.8	8.1	20.8	≤0.05	≤0.05
	LeCompte Bay	8.4	9.1	20.5	≤0.05	≤0.05
	Todds Cove	3.5	9.4	21.2	≤0.05	≤0.05
	Choptank Mouth	0.0	9.8	21.5	≤0.05	≤0.05
12/6/80	Kingston Ldg.	33.3	1.4	22.3	≤0.05	≤0.05
	Frazer Neck	29.5	2.6	22.0	0.30	≤0.05
	Blinkhorn Cr.	22.4	4.8	21.7	0.50	≤0.05
	Goose Pt.	17.7	7.0	21.5	≤0.05	≤0.05
	LeCompte Cr.	8.4	9.5	21.4	0.55	≤0.05
	Todds Cove	3.5	9.9	21.2	0.06	≤0.05
	Choptank Mouth	0.0	10.4	21.2	0.35	≤0.05
25/7/80	Crowberry Cr.	35.9	1.5	27.6	0.72	≤0.05
	Frazer Neck	29.5	4.0	28.4	≤0.05	≤0.05
	Cabin Cr.	21.0	6.3	28.1	0.23	≤0.05
	Goose Pt.	17.7	7.8	28.3	≤0.05	0.08
	Howell Pt.	10.6	10.3	27.5	≤0.05	≤0.05
	Todds Cove	3.5	11.1	27.5	≤0.05	≤0.05
	Choptank Mouth	0.0	11.8	28.2	≤0.05	≤0.05
18/8/80	Marker 60	35.9	1.2	27.9	0.48	1.14
	Marker 55	30.3	4.0	27.4	0.16	0.78
	Marker 41	23.7	6.6	27.0	0.08	0.06
	Marker 31	16.2	9.9	26.7	≤0.05	≤0.05
	Chlora Pt.	7.6	12.2	26.7	≤0.05	0.20
	Todds Cove	3.5	12.8	26.9	0.39	0.95
	Choptank Mouth	0.0	14.2	25.9	≤0.05	≤0.05
16/9/80	Marker 64	38.4	1.3	25.2	≤0.05	0.05
	Kingston Ldg.	33.3	3.4	25.4	0.06	≤0.05
	Frazer Neck	29.5	5.7	25.6	≤0.05	≤0.05
	Cabin Cr.	21.0	10.1	24.6	≤0.05	≤0.05
	Todds Cove	3.5	15.5	24.3	≤0.05	≤0.05
	Choptank Mouth	0.0	17.0	24.1	≤0.05	≤0.05

[a] River mile 0.0 (nautical) taken as being between Blackwalnut and Cook Pts.

Appendix Table 4. Concentrations of atrazine and linuron found in surficial sediments (upper 5 cm) from samples collected along the longitudinal axis of the Choptank River estuary, 1980. Station locations are given in Figure 2.

DATE	LOCATION	RIVER MILE	SEDIMENT HERBICIDE CONCENTRATIONS (ppb)	
			Atrazine	Linuron
25/4/80	Kingston Ldg.	33.3	≤0.1	≤0.1
	Frazer Neck	29.5	≤0.1	≤0.1
	Goose Pt.	17.7	≤0.1	≤0.1
	Choptank Mouth	0.0	≤0.1	≤0.1
12/6/80	Kingston Ldg.	33.3	≤0.1	≤0.1
	Frazer Neck	29.5	≤0.1	≤0.1
	Blinkhorn Cr.	22.4	≤0.1	≤0.1
	Goose Pt.	17.7	≤0.1	0.38
	LeCompte Cr.	8.4	≤0.1	≤0.1
25/7/80	Crowberry Cr.	35.9	≤0.1	≤0.1
	Frazer Neck	29.5	0.13	≤0.1
	Cabin Cr.	21.0	≤0.1	≤0.1
	Goose Pt.	17.7	≤0.1	≤0.1
	Howell Pt.	10.6	≤0.1	≤0.1
	Todds Pt.	3.5	≤0.1	≤0.1
	Choptank Mouth	0.0	≤0.1	≤0.1
18/8/80	Crowberry Cr.	35.9	≤0.1	≤0.1
	Marker "55"	30.3	≤0.1	≤0.1
	Marker "41"	23.7	≤0.1	≤0.1
	Marker "31"	16.2	≤0.1	≤0.1
	Chlora Pt.	7.6	≤0.1	≤0.1
	Todds Pt.	3.5	≤0.1	≤0.1
	Choptank Mouth	0.0	≤0.1	≤0.1
16/9/80	Marker "64"	38.4	≤0.1	≤0.1
	Frazer Neck	29.5	≤0.1	≤0.1
	Choptank Mouth	0.0	≤0.1	≤0.1

Appendix Table 5. Listing of atrazine and linuron concentrations (ppb) measured in the particulate fraction from water samples collected along the longitudinal axis of the Choptank Estuary, 1980. Station locations are given in Figure 2.

DATE	LOCATION	RIVER MILE	VOLUME FILTERED	PARTICULATE HERBICIDE CONCENTRATION (ppb)	
				Atrazine	Linuron
25/4	Frazer Neck	29.5	20ℓ	≤0.1	≤0.1
27/5	Jamaica Point	19.4	20ℓ	≤0.1	≤0.1
27/5	Choptank Mouth	0.0	30ℓ	≤0.1	≤0.1
12/6	Kingston Ldg.	33.3	10ℓ	≤0.1	≤0.1
11/6	Choptank Mouth	0.0	20ℓ	≤0.1	≤0.1
27/7	Crowberry Cr.	35.9	18ℓ	≤0.1	≤0.1
27/7	Choptank Mouth	0.0	20ℓ	≤0.1	≤0.1
18/8	Marker "60"	35.9	20ℓ	≤0.1	≤0.1
18/8	Marker "31"	16.2	20ℓ	≤0.1	≤0.1
18/8	Choptank Mouth	0.0	20ℓ	≤0.1	≤0.1
16/9	Marker "64"	38.4	20ℓ	≤0.1	≤0.1
16/9	Choptank Mouth	0.0	20ℓ	≤0.1	≤0.1
16/9	Frazer Neck	29.5	20ℓ	≤0.1	≤0.1

Appendix Table 6. Concentrations of dissolved atrazine and linuron measured in water samples collected from several locations and dates during 1980 at the Horn Point Experimental watershed. See Fig. 3 for station locations.

DATE	LOCATION	TIME	DISSOLVED HERBICIDE CONCENTRATION, ppb Atrazine	DISSOLVED HERBICIDE CONCENTRATION, ppb Linuron
1/5	Wier	0750	≤0.05	≤0.05
	Wier	0815	5.47	≤0.05
	Wier	0915	4.29	≤0.05
	Wier	1030	2.98	≤0.05
	Wier	1215	2.36	≤0.05
	Wier	1315	≤0.05	≤0.05
	Wier	1545	8.16	≤0.05
	Wier	1615	1.84	≤0.05
	Wier	1845	0.34	≤0.05
	Bridge	0845	18.1	≤0.05
	Bridge	1045	6.24	≤0.05
	Bridge	1245	5.67	≤0.05
	Bridge	1445	5.08	≤0.05
	Bridge	1645	3.29	≤0.05
	Bridge	1905	0.96	≤0.05
	Pond	1200	1.15	≤0.05
	Pond	1430	6.45	≤0.05
	Choptank	1130	≤0.05	≤0.05
2/5	Wier	1605	2.03	≤0.05
	Pond	NR	0.32	≤0.05
	Choptank	NR	≤0.05	≤0.05
22/6	Wier	NR	≤0.05	≤0.05
2/7	Wier	NR	≤0.05	≤0.05
6/7	Wier	1300	≤0.05	≤0.05
8/7	Wier	NR	≤0.05	≤0.05
17/7	Bridge	1800	≤0.05	≤0.05
	Bridge	2200	≤0.05	≤0.05
	Pond	2115	≤0.05	≤0.05
	Choptank	2100	≤0.05	≤0.05
22/7	Wier	2225	0.76	≤0.05
	Wier	2330	0.29	≤0.05
	Pond	2315	0.63	0.05
	Pond	2315	0.52	0.66
23/7	Wier	0100	1.41	≤0.05
	Wier	0145	0.90	≤0.05
	Wier	0800	1.72	0.26
	Wier	1215	≤0.05	0.16
	Pond #1	0010	≤0.05	≤0.05
	Pond #2	0030	1.20	0.09
	Pond #4	0045	0.67	≤0.05
	Pond #5	0030	0.24	≤0.05
	Pond	0015	0.39	≤0.05
	Pond	1315	0.05	≤0.05
	Pond	1400	1.64	1.89
	Pond (mid)	1330	0.21	≤0.05
	Pond (mid)	1832	0.96	2.61
	Pond (mouth)	1825	≤0.05	≤0.05
	Pond (stream)	1843	≤0.05	≤0.05
24/7	Wier	1715	≤0.05	≤0.05
	Pond (mouth)	1615	0.12	≤0.05
	Pond	1630	≤0.05	≤0.05
	Pond	1645	0.19	0.07

DATE	LOCATION	TIME	DISSOLVED HERBICIDE CONCENTRATION, ppb Atrazine	DISSOLVED HERBICIDE CONCENTRATION, ppb Linuron
4/8	Wier	1430	≤0.05	≤0.05
5/8	Wier	0800	≤0.05	≤0.05
12/8	Wier	1000	≤0.05	≤0.05
21/8	Wier	0830	≤0.05	≤0.05
26/8	Wier	1500	≤0.05	≤0.05
10/9	Wier	0715	0.58	0.29
24/7	Wier	1715	≤0.10	≤0.10
	Pond	1615	≤0.10	≤0.10
	Pond	1630	≤0.10	≤0.10
	Pond	1645	≤0.10	≤0.10
4/8	Wier	1430	≤0.10	≤0.10
12/8	Wier	1000	≤0.10	≤0.10
26/8	Wier	1500	≤0.10	≤0.10
5/9	Wier	0800	≤0.10	≤0.10
10/9	Wier	0715	≤0.10	≤0.10
19/9	Wier	0930	≤0.10	≤0.10
26/9	Wier	1000	≤0.10	≤0.10
13/10	Wier	1000	≤0.10	≤0.10
16/10	Wier	1900	≤0.10	≤0.10
24/10	Wier	NR	≤0.10	≤0.10
31/10	Wier	NR	≤0.10	≤0.10
14/11	Wier	NR	≤0.10	≤0.10
26/11	Wier	NR	≤0.10	≤0.10
19/9	Wier	0930	0.75	≤0.05
26/9	Wier	1000	≤0.05	≤0.05
3/10	Wier	1000	≤0.05	≤0.05
16/10	Wier	1900	0.62	0.83
24/10	Wier	NR	≤0.05	≤0.05
31/10	Wier	NR	≤0.05	≤0.05
14/11	Wier	NR	0.87	≤0.05
26/11	Wier	NR	≤0.05	≤0.05

Appendix Table 7. Concentrations of atrazine and linuron in the suspended particulate fraction of water samples collected from several monitoring stations at the Horn Point Experimental watershed, 1980. See Fig. 3 for station locations. NR=not recorded.

DATE	LOCATION	TIME	SUSPENDED PARTICULATE HERBICIDE CONCENTRATION, ppb	
			Atrazine	Linuron
1/5	Wier	0815	≤0.10	≤0.10
		1000	≤0.10	≤0.10
		1300	≤0.10	≤0.10
		1845	≤0.10	≤0.10
		NR	≤0.10	≤0.10
		NR	≤0.10	≤0.10
	Bridge	0845	≤0.10	≤0.10
		1100	≤0.10	≤0.10
		1445	≤0.10	≤0.10
		1805	≤0.10	≤0.10
		NR	≤0.10	≤0.10
	Pond	1200	≤0.10	≤0.10
		1430	≤0.10	≤0.10
		1500	≤0.10	≤0.10
	Choptank	1130	≤0.10	≤0.10
2/5	Wier	1605	≤0.10	≤0.10
	Pond	1500	≤0.10	≤0.10
		NR	≤0.10	≤0.10
	Choptank	1400	≤0.10	≤0.10
18/5	Wier	0730	≤0.10	≤0.10
27/5	Choptank	NR	≤0.10	≤0.10
1/6	Wier	1230	≤0.10	≤0.10
3/6	Wier	1800	≤0.10	≤0.10
22/6	Wier	NR	≤0.10	≤0.10
2/7	Wier	NR	≤0.10	≤0.10
8/7	Wier	NR	≤0.10	≤0.10
16/7	Wier	1300	≤0.10	≤0.10
17/7	Wier	0010	≤0.10	≤0.10
	Wier	2215	≤0.10	≤0.10
	Bridge	2200	1.36	≤0.10
	Pond	2115	≤0.10	≤0.10
	Choptank	2100	≤0.10	≤0.10
22/7	Wier	2225	≤0.10	≤0.10
	Pond	2315	≤0.10	≤0.10
	Pond	2330	≤0.10	≤0.10
23/7	Wier	0100	≤0.10	≤0.10
	Wier	0145	≤0.10	≤0.10
	Wier	0800	≤0.10	≤0.10
	Wier	1215	≤0.10	≤0.10
	Wier	1742	≤0.10	≤0.10
	Pond	0015	≤0.10	≤0.10
	Pond	0030	≤0.10	≤0.10
	Pond (mid)	0030	≤0.10	≤0.10
	Pond	0045	≤0.10	≤0.10
	Pond (mouth)	1315	≤0.10	≤0.10
	Pond (mid)	1330	≤0.10	≤0.10
	Pond	1400	≤0.10	≤0.10
	Pond	1832	≤0.10	≤0.10
	Pond (mouth)	1832	≤0.10	≤0.10
	Pond (stream)	1843	≤0.10	≤0.10

Appendix Table 8. Concentrations of atrazine and linuron in soil samples collected at the Horn Point Experimental watershed, 1980.

DATE	LOCATION	HERBICIDE CONCENTRATION, ppb	
		Atrazine	Linuron
6/7	crop soil, HPEL[a]	≤0.10	≤0.10
8/7	crop soil, HPEL	≤0.10	≤0.10
4/8	crop soil, HPEL	0.26	≤0.10
12/8	crop soil, HPEL	1.20	≤0.10
21/8	crop soil, HPEL	0.79	≤0.10
26/8	crop soil, HPEL	1.10	≤0.10
5/9	crop soil, Wier	≤0.10	≤0.10
10/9	crop soil, Wier	≤0.10	≤0.10
19/9	crop soil, HPEL	≤0.10	≤0.10
26/9	crop soil, HPEL	≤0.10	≤0.10
3/10	crop soil, Wier	≤0.10	≤0.10
24/10	crop soil, Wier	≤0.10	≤0.10
31/10	crop soil, HPEL	≤0.10	≤0.10
14/11	crop soil, Wier	≤0.10	≤0.10
26/11	crop soil, HPEL	≤0.10	≤0.10

[a] HPEL referens to the Horn Point Experimental watershed.

Appendix Table 9. Concentrations of dissolved atrazine and linuron in water collected from several locations at the Horn Point Experimental watershed and from several stations along the Choptank River estuary in 1981. See Figs. 1-3 for station locations.

LOCATION	DATE	TIME	DISSOLVED HERBICIDE CONCENTRATION, ppb	
			Atrazine	Linuron
Horn Point Experimental Watershed				
Weir	5/10	1812	0.81	≤0.05
	5/11	0905	1.59	≤0.05
		1330	2.00	2.37
	5/12	0730	2.33	≤0.05
		NR	7.02	≤0.05
	5/15	1800	13.72	1.88
	5/29	NR	0.78	3.02
	6/2	1030	10.07	1.79
	6/8	1330	≤0.05	≤0.05
	6/10	0820	≤0.05	≤0.05
	6/15	1130	0.66	0.45
	6/19	1330	≤0.05	≤0.05
	6/20	1700	12.99	1.88
	6/22	1100	2.48	16.55
	7/13	1000	1.62	≤0.05
	7/20	1100	≤0.05	≤0.05
	7/22	0930	≤0.05	≤0.05
Bridge	5/15	NR	4.16	≤0.05
	5/15	1800	45.05	2.31
Pond (mid)	5/11	1830	1.16	≤0.05
(mid)		1830	5.38	≤0.05
(mid/surface)		1830	8.08	≤0.05
(mouth)		1830	1.79	4.03
Pond (mid)	5/13	NR	0.99	≤0.05
(mid/surface)	5/15	NR	17.04	0.93
(mid/bottom)		NR	1.56	1.56
(mid)	7/22	1000	≤0.05	≤0.05
(mouth)		1000	≤0.05	≤0.05
(mouth)		1000	≤0.05	≤0.05
Beaver Dam				
	5/5	NR	3.02	≤0.05
	5/12	2000	9.00	≤0.05
	5/21	NR	2.60	≤0.05
	6/2	NR	1.12	1.42
	6/11	NR	0.96	≤0.05
	6/18	NR	1.18	0.34
	7/23	NR	≤0.05	≤0.05
Choptank River Stations				
Beach Haven	5/10	NR	2.25	≤0.05
Tuckahoe Cr	5/13	0840	5.94	1.96
Dover Br	5/13	NR	1.79	2.42
Todds Cove	5/13	1830	≤0.05	2.48
Bouy 12B	5/13	1800	≤0.05	0.76
Castle Haven	5/13	1930	0.19	2.19
Horn Point	7/22	1830	≤0.05	≤0.05
Benoni Pt	7/22	1650	0.13	≤0.05
Castle Haven	7/22	1700	0.61	≤0.05
Todds Cove	7/22	1540	≤0.05	≤0.05
Nelson Pt	7/22	1630	≤0.05	≤0.05
Beach Haven	7/23	NR	≤0.05	≤0.05
Tuckahoe Ldg	7/23	NR	0.87	≤0.05
Dover Br	7/23	NR	1.15	≤0.05

QUALITY ASSURANCE DATA

Appendix Table 10. Summary of duplicate field analyses of water.

STATION	1980 DATE	HERBICIDE CONC. (ppb)	
		ATRAZINE	LINURON
SI-5	24/4	0.15 0.20	<0.05 <0.05
SI-1	23/4	0.30 0.34	<0.05 <0.05
SI-4	13/6	1.09 1.05	<0.05 <0.05
SI-19	12/6	0.10 0.13	<0.05 <0.05
SI-4	26/7	0.20 0.21	0.80 0.84
SI-1	29/7	0.13 0.10	0.25 0.20
SI-1	22/9	0.93 0.89	0.31 0.34
SI-4	18/9	<0.05 <0.05	<0.05 <0.05
Jamaica Pt	27/5	0.06 0.05	<0.05 <0.05
Marker 41	18/8	0.08 0.08	0.06 0.05
Patuxent	13/7	0.81 0.81	<0.05 <0.05

QUALITY ASSURANCE DATA

Appendix Table 11. Summary of spiked sample analyses and percent recovery.

STATION	1980 DATE	HERBICIDE CONC. (ppb) ATRAZINE	HERBICIDE CONC. (ppb) LINURON	% RECOVERY AT.	% RECOVERY LIN.
SI-1	23/4	0.15	<0.05	-	-
+2 ppb spike	23/4	2.09	1.98	97.2	99.0
SI-1	16/6	<0.05	<0.05	-	-
+2 ppb spike	16/6	2.01	2.03	100.5	101.5
SI-1	29/7	0.13	0.25	-	-
+2 ppb spike	29/7	2.01	2.08	94.4	92.4
SI-1	22/9	0.93	0.31	-	-
+2 ppb spike	22/9	3.01	2.18	102.7	94.4
Wier	22/7 (2225)	0.76	<0.05	-	-
+2 ppb spike	22/7 (2225)	2.59	1.93	93.8	96.5
Wier	4/8 (1430)	<0.05	<0.05	-	-
+2 ppb spike	4/8 (1430)	2.05	1.97	102.5	98.5
Wier	3/10 (1000)	<0.05	<0.05	-	-
+2 ppb spike	3/10 (1000)	1.91	2.03	95.5	101.5

CHAPTER II

TEMPORAL RESPONSES OF A SUBMERGED VASCULAR PLANT AND ITS ASSOCIATED AUTOTROPHIC COMMUNITY TO ATRAZINE STRESS IN ESTUARINE MICROCOSMS

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ABSTRACT

Experiments were conducted over eight weeks in large (700 1) estuarine microcosms to test a variety of physiological and morphological responses of the submerged vascular plant, *Potamogeton perfoliatus*, to the herbicide, atrazine. At atrazine concentrations of about 0.13 ppm (equivalent to highest values occurring in the estuary) significant decreases in apparent O_2 production (P_a) were exhibited immediately following treatment; however, a significant trend of photosynthetic recovery was evident within 2 wk, eventhough atrazine levels remained relatively constant. Since herbicide treatment exerted negligible effect on dark respiration (R_n), the metabolic ratio $P_a:R_n$ responded in much the same way as did P_a. Integrated community production and consumption of O_2 followed patterns similar to those for *P. perfoliatus*, and on hypothesized atrazine-induced shift in relative contributions of each autotrophic group (macrophytes, phytoplankton, epiphytes benthic microalgae) was not borne out. While areal densities of plant shoots for the experimental populations were unaffected by this level of treatment, total biomass decreased significantly, and a lag of 2-4 wk was evident following initial losses of P_a. Morphology of individual shoots was markedly influenced by atrazine, with significant increases in mean shoot length and decreases in weight per unit length. Furthermore, chlorophyll *a* per unit leaf area increased 5-fold with atrazine treatment, and Such effects are analogous to previously reported shade adaptations of this and other submerged plants. The implications of these results are discussed in terms of design for further dose-response studies of herbicides and SAV.

INTRODUCTION

Structural and functional attributes of submerged vascular plants provide a basis for diverse ecosystems which dominate the littoral zones of many aquatic environments. These plants, which are among the most productive in the world (Westlake, 1968; Mann 1982), supply a food-base for variegated detrital trophic chains. Their physical structure creates a unique habitat for epiphytic organisms (Nagel, 1968; Marsh, 1973; Nelson, 1979) and a refuge for small fish and invertebrates from predation (Adams, 1976; Coen et al., 1981). Submerged aquatic vegetation (SAV) markedly influences several aspects of its environment, including: the local physical circulation and related sedimentological processes (Ginsburg and Lowenstam; Ginsburg, 1958; Fonseca et al. 1982; Harlin et al. 1982). The cycling of nutrients (McRoy et al. 1972, Howard-Williams, 1981), and the geochemistry of sediment pore-waters (Iizumi et al. 1980, Kenworthy et al. 1982).

While the ecological importance of SAV is widely recognized, in many aquatic systems this role is being diminished or lost as plant populations undergo major declines in abundance (e.g. Den Hartog and Poldermann, 1975; Peres and Picard, 1975). In one large North American estuary, Chesapeake Bay, a continuing decrease in SAV distribution and abundance has been apparent since the mid-1960's (Bayley et al. 1978, Orth and Moore 1981). Numerous factors have been suggested as contributing to this and other SAV declines, including decreased water clarity; (e.g. Kullberg, 1974; Jupp and Spence 1977); nutrient enrichment and associated algal growth (Phillips et al., 1978; Sand-Jensen and Søndergaard, 1981); and intense, localized phenomenon such as grazing and foraging by waterfowl and fish (Orth 1975; Kiørboe 1980).

One recent study has suggested that inadvertent runoff of agricultural herbicides (primarily the widely used compound, atrazine) may have contributed to the loss of SAV in Chesapeake Bay (Correll and Wu 1982). Moreover, in some watercourses dense populations of these plants have been perceived as deleterious to human interests, and active application of herbicides has been used to control SAV growth (e.g., Brooker and Edwards 1975, Robson and Barrett 1977). Thus, there is a need to understand the nature and magnitude of herbicide effects on SAV. Several studies have examined herbicide effect on SAV by monitoring either loss of photosynthesis in shortterm (2h-4d) exposures (Sutton et al. 1969, Bielecki and Skrabka 1976) or changes in growth, mortality and/or photosynthesis after longer (3-6 wk) exposures (Forney and Davis 1981, Correll and Wu 1982). However, few investigations have considered temporal patterns of response, and other measures of SAV physiology (such as pigmentation and morphology) have largely been overlooked in relation to herbicides. Some researchers have measured herbicide effects on photosynthesis and respiration of integrated SAV communities (Brooker and Edwards 1973 a,b, Strange 1976, Strange and Shreck 1976), but none have measured effects at levels of individual plants, plant populations, and associated communities.

The present study was initiated to investigate temporal trends in photosynthesis, respiration, biomass and abundance (as well as other morphological and physiological variables) for the submerged plant, Potamogeton perfoliatus, in response to atrazine stress over several weeks. Broader, ecological effects of atrazine were also considered in terms of oxygen production and respiration for whole experimental communities and for major autotrophic groups comprising those communities. The overall goal of this study was to characterize atrazine effects on a spectrum of plant and community physiological and ecological variables and to determine temporal patterns of these effects. This study was not intended to develop detailed dose-response relationships for atrazine and P. perfoliatus; these are provided elsewhere (Kemp et al. 1982).

MATERIALS AND METHODS

Experimental Design

We hypothesized that indications of stress in P. perfoliatus would be evident within 1-3 weeks following atrazine treatment in terms of several physiological variables, including photosynthesis, respiration, pigmentation, stem and leaf weight and meristic relations. We also postulated that some recovery would occur within weeks, and that other autotrophic groups would exhibit responses inverse to those for SAV due to competitive interaction. Since the objectives of this study were to discern the qualitative nature of stress responses, we utilized relatively high concentrations of atrazine (approximating extreme in situ conditions) to increase the likelihood of observable effects.

Time-series observations of SAV responses required experimental populations large enough to allow weekly removal of several plants without markedly disturbing the population (<5% removal). Plant growth (shoot length and density) was measured non-destructively at weekly intervals to minimize sampling effects; however, periodic removal of plants was also necessary to establish length-weight relations. The large experimental microcosms used here provided sufficient plant material for the above measurements,and adequate water volume and surface area were also available in these systems for growth of associated micro-algal populations.

A split-plot, 2-way analysis of variance was employed for statistical interpretation, with herbicide dosage as the main plot and time and replicates as subplots. In this factorial experiment significant differences ($p<0.05$) among means were isolated with the Student-Newman-Kuels multiple range test (Snedecor and Cochran 1967).

Experimental Systems

Microcosm communities were established in 6 large glass aquaria (1.8 x 0.6 x 0.8 m), each containing approximately 700 ℓ filtered (5μ) water and 12 cm depth of sediments from the Choptank River estuary, a tributary on the eastern shore of Chesapeake Bay. The sandy-silt sediment, which was obtained from an existing SAV bed, had sand/silt/clay proportions of 76/20/4 and contained about 0.8% organic matter. Microcosms were maintained in an air-conditioned laboratory and water temperature remained relatively constant throughout the study (22-26 C), with typical diel ranges of 2 C. An initial salinity of 9.5°/∘∘ increased about 1°/∘∘ from beginning to end of experiment due to evaporation. The microcosms were illuminated by banks of "cool white" fluorescent lights on a 14 h photoperiod. Water in the microcosms was continuously circulated using centrifugal pumps (Little Giant Model 2 MD, rated flow of 17 ℓ min^{-1}), with inlet and outlet diffusers separated at opposite ends of the tanks. Each microcosm was seeded initially with 75 individual plants (shoots 6-50 cm, rhizomes 2-5 cm) of the submerged vascular plant, *Potamogeton perfoliatus*, obtained in July 1978 from a population in the Choptank River estuary.

Atrazine Addition and Analysis

After establishing the microcosm communities, a period of 3 weeks was allowed for these systems to equilibrate prior to additon of atrazine. In an effort to simulate actual runoff conditions, atrazine was added to microcosms adsorbed onto sediments. Technical grade (96.4% purity) atrazine was dissolved in 100 mℓ acetone and dried onto a 1.0 kg (dry weight) sediment sample. Aquaria were randomly paired and selected for each of 3 treatments: high dosage (860 mg atrazine); low dosage (86 mg atrazine) and control (sediment addition only). The herbicide and/or sediment dosages were introduced by vigorously mixing into the microcosm water. Water, sediment and suspended solids (>0.3 μ) were collected for analysis of atrazine residues at 3 times during the experiment (1 d prior to treatment, and 36 h and 30 d following treatment). Atrazine residues were extracted in methanol (a Soxhlet apparatus was used for sediments) and analyzed using gas chromatographic techniques (Purkayastha and Cochrane 1973). Analyses were performed at EnCase Laboratories (Greensboro, NC, USA) through the cooperation of Ciba-Geigy Corporation.

Photosynthesis and Respiration

Photosynthesis and respiration of *P. perfoliatus* were measured in each microcosm at 6-8 d intervals beginning 1 wk prior to treatment. Individual plants were carefully removed from sediments with minimum disruption, and a single shoot with about 5 cm length of rhizome (roots intact) was washed free of sediments and epiphytes and placed into a BOD bottle (300 mℓ) containing ambient water from the respective microcosm. Apparent photosynthesis (P_a) and dark respiration (R_n) were measured as oxygen (O_2) changes during incubations of 2 h in 6 replicate clear and opaque bottles suspended at the depth of the plant canopy in each system (Buesa 1975). Epiphytes removed from individual plants were incubated separately in light and dark (L and D) BOD bottles, and triplicate L and D bottles containing only water and suspended material were incubated to estimate plankton production and respiration (used also as a "blank" to correct plant and epiphyte measurements). Metabolism of the benthic community was determined using L and D acrylic cylinders (800 mℓ) inserted 2 cm into sediments. In all cases rates were estimated as changes in O_2 measured at 2-3 h intervals over a 9-12 h incubation. Bottles were suspended in front of the circulation pump outlet diffuser to gently and continually mix them, and bottles were shaken by hand at 30 min intervals to insure that gas diffusion gradients would be minimized (e.g., Conover 1967, Westlake 1978).

At the end of each incubation, plants were placed in aluminum foil dishes and dried at 60 C to constant weight, to standardize metabolic rates to dry weight of plant material (g O_2 (g d.w.)$^{-1}$ h^{-1}). Plant and epiphyte production and respiration rates were also extrapolated to g O_2 m^{-2}d^{-1} by multiplying specific rates by plant density and photoperiod, while water depth (or height under chamber) was used to obtain areal rates for plankton and benthos. Polarographic oxygen electrodes (Orbisphere Model 2709) were used to measure O_2 concentrations, and air calibration was performed daily. The potential problems of lacunal storage of O_2 in submerged vascular plants (e.g., Zieman and Wetzel 1980) were not encountered in these well circulated systems (Westlake 1978), and periodic contemporaneous measurements of ^{14}C-uptake (Lewis et al., 1982) yielded photosynthetic quotients (O_2 production:^{14}C incorporation) of about 1.5 (Lewis, 1980).

Community metabolism (P_a and R_n) was also monitored weekly by measuring mean O_2 concentrations in the open water of each microcosm at the beginning and end of the photoperiod. Production and respiration of microcosm communities were taken as the increase and decrease, respectively, in O_2 concentration between successive dawn and dusk measurements (Odum, 1956). Concentrations of O_2 were maintained above 3.5 ppm throughout the experiment by periodic aeration using standard aquarium bubblers. Corrections for gas transfer across the air-water interface were made by applying a diffusion coefficient to the observed mean saturation deficit (Odum and Hoskins, 1958). Coefficients for O_2 diffusion (averaging 0.15 g O_2 m^{-2}h^{-1}atm^{-1}) were obtained by monitoring the rate of O_2 invasion into microcosms which had been previously deoxygenated by bubbling with nitrogen gas (Kemp et al., 1983).

Plant Abundance, Biomass and Morphometry

Plant density (shoots m^{-2}) and shoot length were determined each week beginning 2 wk prior to treatment. Quadrats in each microcosm were carefully marked, and all stems in each were counted and measured for length during a given sampling. Plant biomass in the aquaria was estimated with the following non-destructive technique. At the beginning and end of the experiment, and twice during the study, 20-30 plants representative of the entire spectrum of size-classes, were selected from each experimental treatment. Plants were measured individually for length and number of leaves and were dried to constant weight (at 60 C). Biomass of each microcosm was estimated for each week using linear regressions of weight versus length and applying measurements of mean and total length to obtain total plant weight. At the conclusion of the experiment, 3 replicate cores (9 cm dia) were taken from microcosm communities to determine representative ratios of above-ground:below-ground plant biomass, and total aboveground (shoot) material was harvested, dried and weighed.

Other Variables

Chlorophyll *a* concentrations in *P. perfoliatus* were measured at the end of the experiment by taking leaf sections (18 mm^2) in duplicate from the uppermost leaves of 3 plants in microcosms. Each leaf disc was extracted in the dark at 5 C for 24 h in 90% acetone after tissue grinding (Vernon, 1960). Absorbance values were read at 665 and 745 nm on a Beckman (Model 88) spectrophotometer, and chlorophyll concentrations were calculated using the trichromatic equations given by Strickland and Parsons (1972). Plankton and benthic algal pigments were estimated according to the methods of Strickland and Parsons (1972).

Photosynthetically active radiation (PAR) was measured weekly in each microcosm at mid-water depth using a LICOR (Model LI-192SB) cosine-corrected sensor. Total suspended solids were also measured by filtering triplicate 250 mℓ water samples through pre-weighted, dried Whatman GFC (1.2 μ) filters, which were dried to constant weight at 90 C, cooled in dessicator and reweighed (Strickland and Parsons, 1972). The filtrate was frozen for subsequent analyses of nitrate, nitrite and orthophosphate using methods of Strickland and Parsons (1972), while ammonium was analyzed as in Grasshoff and Johannsen (1972). Temperature and salinity were monitored at each measurement of microcosm O_2 concentrations (weekly at dawn-dusk-dawn) using a Beckman (Model RS5-3) induction salinometer and thermistor.

RESULTS AND DISCUSSION

Atrazine Concentrations and Partitioning

Aqueous concentrations of atrazine were relatively constant throughout the experiment following treatments, with values of about 0.13 ppm for "low" dose systems and 1.20 ppm for "high" dose. The lower concentrations correspond to the maximum concentrations of atrazine observed in Chesapeake Bay (Kemp et al., 1982). Atrazine concentrations were similar at 3 and 30 d following treatment in both high and low dose experimental systems, and atrazine budgets developed for these microcosm, indicate about 15-20% loss of the herbicide during the experiment. Assuming a first-order degradation process, this implies a half-life of about 3-4 mo, which is 3-6 times that estimated by Jones et al. (1982) under different experimental conditions (exposed to natural ultraviolet radiation). By far, the vast majority of the compound remained in solution, with about 11, 6 and 0.03% of the total mass contained in sediments, plants and suspended solids, respectively (Fig. 1). The estimated plant uptake of atrazine was similar to that reported by previous investigators (Funderbunk and Lawrence, 1963; Negi et al., 1963; Sutton et al., 1969).

Environmental Variables

During the course of the experiment there were several notable changes in the milieu of these microcosms. Some of these changes are evident in control systems, and probably reflect natural processes in the development of microcosm communities. For example, total seston (suspended particulates) underwent a significant decrease in concentration from the beginning to end of the study but showed no response to treatment (Table 1). While cylces of fluctuating concentrations of dissolved nutrients may be evident on longer time-scales (Kemp et al., 1981), during the 7-8 wk of this study control and low dose microcosms experienced only small changes in nutrients (Table 1). Under high

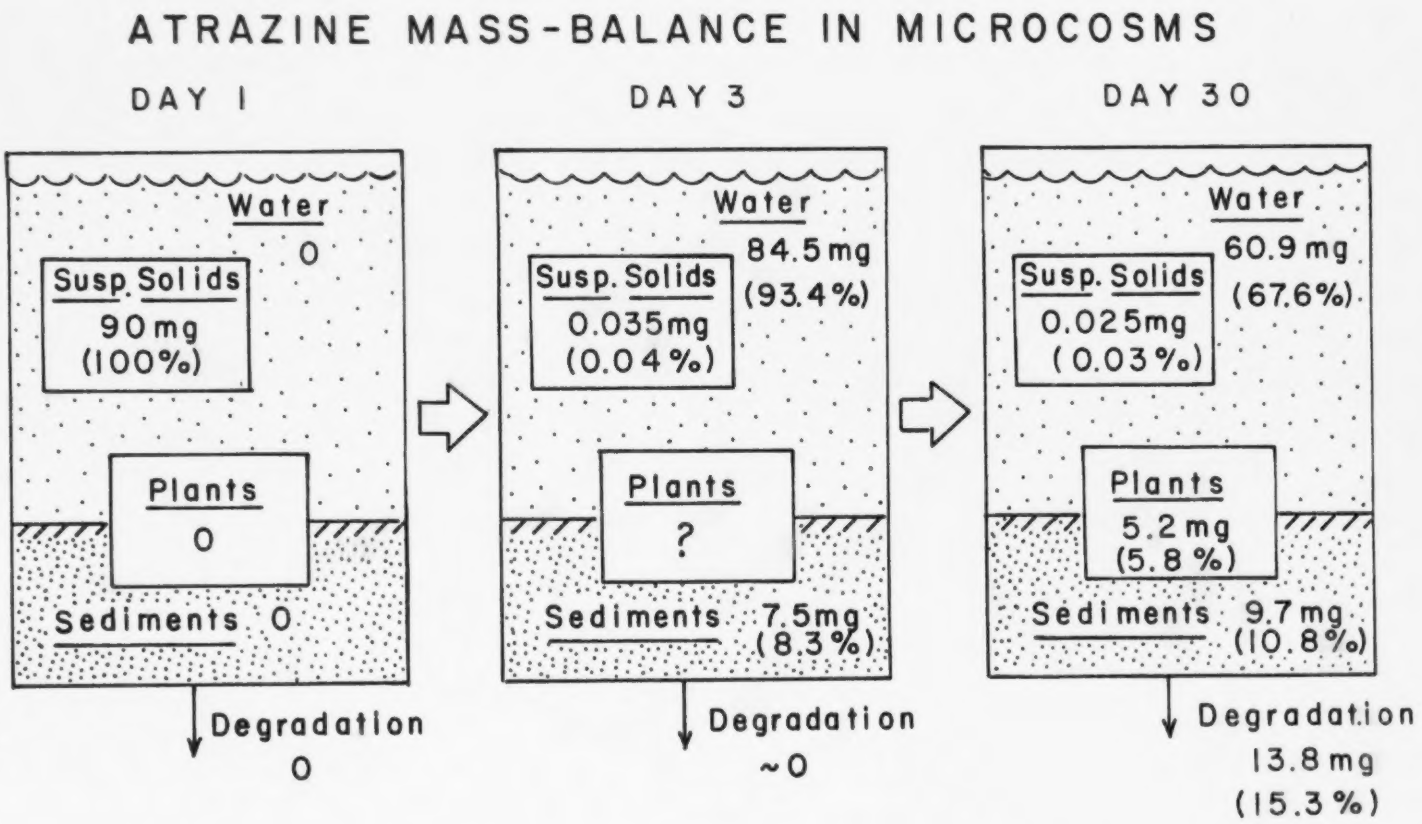
ATRAZINE MASS-BALANCE IN MICROCOSMS
DAY 1
Water
0
Susp. Solids
90 mg
(100%)
Plants
0
Sediments 0
Degradation
0
DAY 3
Water
84.5 mg
(93.4%)
Susp. Solids
0.035mg
(0.04%)
Plants
?
Sediments 7.5mg
(8.3%)
Degradation
~0
DAY 30
Water
60.9 mg
(67.6%)
Susp. Solids
0.025mg
(0.03%)
Plants
5.2 mg
(5.8%)
Sediments 9.7 mg
(10.8%)
Degradation
13.8 mg
(15.3%)

Fig. 1. Estimated mass-balance for atrazine in estuarine microcosms at 1, 3 and 30 d following treatment of 86 mg (low dose) of herbicide.

TABLE 1. Summary of selected environmental variables for microcosm communities treated with the herbicide, atrazine.*

Variable	Treatment					
	Control		Low		High	
	Pre	Post	Pre	Post	Pre	Post
Total Seston (mg/ℓ)	27.8 ±0.2	19.2 ±5.1	27.5 ±1.9	21.3 ± 5.5	29.4 ±1.4	20.3 ±5.0
PAR (μEin $m^{-2}s^{-1}$)	-	122 ±4	-	131 ±10	-	131 ±8
NO_3^- (μM)	0.46	0.65 ±0.11	0.35	1.13 ±0.46	0.41	11.2 ±1.30
NO_2^- (μM)	0.22	0.12 ±0.05	0.35	0.23 ±0.10	0.12	1.44 ±0.41
NH_4^+ (μM)	0.21	0.93 ±0.15	2.32	2.07 ±0.14	0.30	40.21 ±11.8
$PO_4^{\equiv}$ (μM)	0.17	0.14 ±0.02	0.32	0.08 ±0.02	0.191	0.63 ±0.21

*Given are means ± standard deviation for 2 pre-treatment samplings and for samplings in the final 2 weeks of post treatment observation. For replication, n=4 for seston, nutrients and post-treatment PAR, n=1 for pretreatment nutrients.

#Photosynthetically active radiation measured at mid depth of water column (ca. 35 cm below water surface).

herbicide treatments there were marked increases in concentration for all nutrient species measured (NO_3^-, NO_2^-, NH_4^+, $PO_4^=$), resulting from plant excretion and decomposition (e.g., Strange, 1976). Although light available for plant photosynthesis (PAR) was below saturation values (Goldsborough, 1983), there were no significant differences among treatments (Table 1).

Plant Photosynthesis and Respiration

Over the first 4 wk of measurements apparent photosynthesis (P_a) for control plants (*P. perfoliatus*) exhibited a continual increase (0.41 to 1.11 $gO_2m^{-2}d^{-1}$), with a significant decline (to 0.67 $gO_2m^{-2}d^{-1}$) in the last week (Fig. 2a). Values of P_a normalized per unit weight of plant did not change significantly through week 6 (mean = 2.79 mg O_2 g dry $wt.^{-1}h^{-1}$), with a sharp decline again in week 7 (1.75). Under low atrazine dosage P_a decreased significantly with treatment in week 4 followed by a steady and significant recovery (Fig. 2a). While P_a for low dose microcosms remained below that for controls throughout post-treatment observations, by week 7 it was statistically indistinguishable from pre-treatment values (week 3). Furthermore, P_a per unit weight was not significantly different for control and low dosed plants in the final week of observation. At high herbicide dosage P_a remained negative throughout. This recovery of P_a at low dose is remarkable in view of the fact that atrazine concentrations remained relatively constant throughout. To our knowledge, there have been no previous reports of this behavior, and the mechanism whereby it might occur is uncertain (Kemp et al. 1982).

The mean losses of P_a over the 4 wk following treatment for plants exposed in this study to low (0.13 ppm) and high (1.2 ppm) dosage of atrazine were 100% and about 50% compared to control plants, respectively. These results are comparable to previous reports on effects of atrazine and related herbicides on SAV photosynthesis. In their investigation of simazine effects on 3 freshwater vascular plants, Sutton et al. (1969) observed a maximum of 40% reduction in O_2 concentration (rather than rate of production) after 4 d at 0.12 ppm. Similarly, Bielecki and Skrabka (1976) reported about 35% loss of carbon dioxide fixation by the macrophyte, *Spirodela polyrrhiza*, exposed to 0.2 ppm simazine for 24 h, while Fowler (1977) observed about 50% decrease in P_a of *Myriophyllum* sp. at 0.5 ppm of another *s*-triazine (DPX 2674) after 10 d. In a study of longer duration, Correll and Wu (1982) reported P_a for 4 SAV species (*P. pectinatus*, *Z. palustris*, *V. americana* and *Zostera marina*) after 3-6 wk exposures to 0.075 and 0.65 ppm atrazine. At the lower concentration effects on P_a ranged from a 48% stimulation to a 39% depression compared to controls, while the higher concentration resulted in 71-100% loss of P_a. There was no evidence of metabolic recovery in any of the short-term experiments, and data provided in the longer study of Correll and Wu (1982) was insufficient for any such interpretation.

The pronounced effects of atrazine and other *s*-triazine herbicides on SAV photosynthesis is to be expected in view of the fact that the mode of toxic action involves Hill Reaction blockage on the reducing side of photosystem II (Souza-Machado et al., 1978). However, the anticipated effects on plant respiration are, perhaps, less obvious. Dark respiration (R_n) was unaffected by

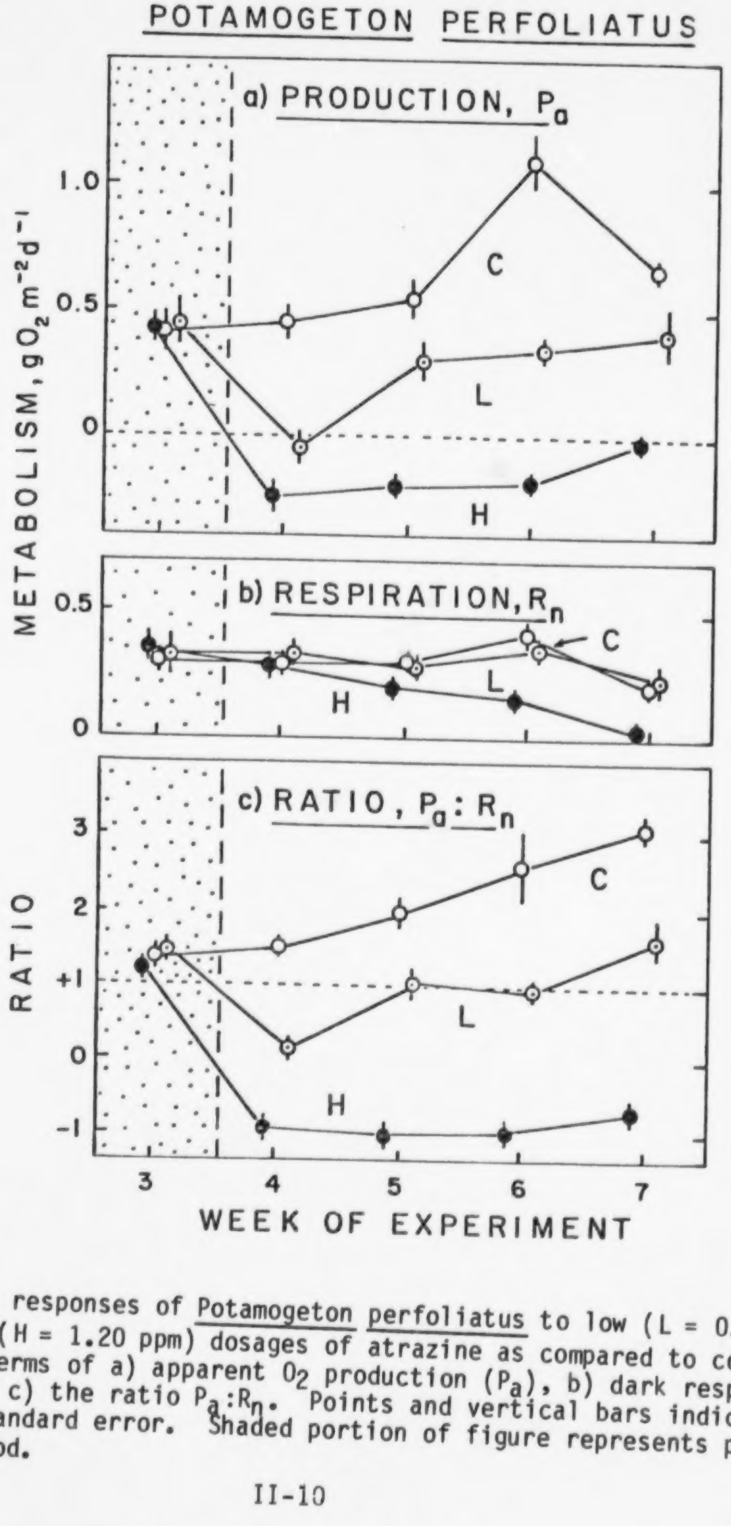

Fig. 2. Metabolic responses of Potamogeton perfoliatus to low (L = 0.13 ppm) and high (H = 1.20 ppm) dosages of atrazine as compared to controls (C), in terms of a) apparent O_2 production (P_a), b) dark respiration (R_n), and c) the ratio $P_a:R_n$. Points and vertical bars indicate mean ± standard error. Shaded portion of figure represents pretreatment period.

low atrazine treatment, remaining relatively constant throughout the study. Under high dosage R_n showed a steady and significant decline through week 7 (Fig. 2b). The ratio of P_a to dark respiration provides an index of the metabolic energy balance for the plant population (e.g., Drew 1979). Since R_n changed very little, patterns of $P_a:R_n$ (on a diel basis) were similar to the temporal and treatment trends for P_a alone (Fig. 2c). Thus, for the recovery period (weeks 5-7) low dosed plants were able to undergo minimal net growth, as $P_a:R_n$ remained near 1.0 for weeks 5-6, increasing somewhat to 1.6 in week 7.

Plant Abundance and Selected Morphological and Physiological Variables

Responses of plant photosynthesis and respiration to herbicide treatment were eventually manifested as changes in abundance and biomass of these SAV populations, as well as changes in plant morphology. Density ($\#/m^2$) of shoots (stems with leaves) increased in a nearly linear manner between weeks 2 and 6 for both control and low dose microcosms, with no change occurring from week 6 to 7 (Fig. 3a). There was no significant difference between these two treatments. At high atrazine dosage (1.2 ppm) a significant decline was observed from week 5 to 7, with final shoot density only about 20% of control values (Table 2). While changes in shoot numbers provided only weak indication of herbicide response here for *P. perfoliatus*, Correll and Wu (1982) found shoot mortality to be a reasonable measure of stress for *V. americana* treated with relatively low concentrations of atrazine (0.012 ppm).

At the beginning and throughout the experiment, representative samples of plant shoots were obtained from microcosms to establish length (L, cm) versus weight (B, gm) relationships, so that stem density data could be translated into biomass. Significant linear regressions were obtained throughout; however, significant changes in slope ($p<0.05$, analysis of covariance) were also observed. For pre-treatment weeks the regression for plants in all microcosms was

$$B = 0.0032\ L + 0.0003 \qquad (r^2 = 0.89).$$

The slope of this relation for the pooled control system data for weeks 5 and 7 (Fig. 4) had increased significantly (indicating that a unit length of plant was heavier). The slope of the L-B regression for low dosed plants was significantly less than that for controls in the final 3 wk (Fig. 4), and the mean L:B ratio was significantly increased with treatment (2.4, 4.3 and 6.1, respectively, Table 2).

Applying the regressions in Fig. 4 to the stem density data in Fig. 3a, we obtain an estimate of plant biomass for *P. perfoliatus* populations in experimental microcosms (Fig. 3b). In control microcosms through week 6, net accretion of plant biomass proceeded at a consistent rate (0.7 g d.w. $m^{-2}d^{-1}$) which is identical to the mean value of post-treatment P_a (assuming that g O_2:g d.w. = 1.0 as in Odum, 1971). Significant effects of atrazine dosage on total plant biomass were detectable 2 wk following dosage for both treatments (Fig. 3b). Thus, there was a 1 wk lag between the initial indication of metabolic decline and incipiently observable losses of plant material. By week 7 biomass in the high dose microcosms had been virtually eliminated, as a

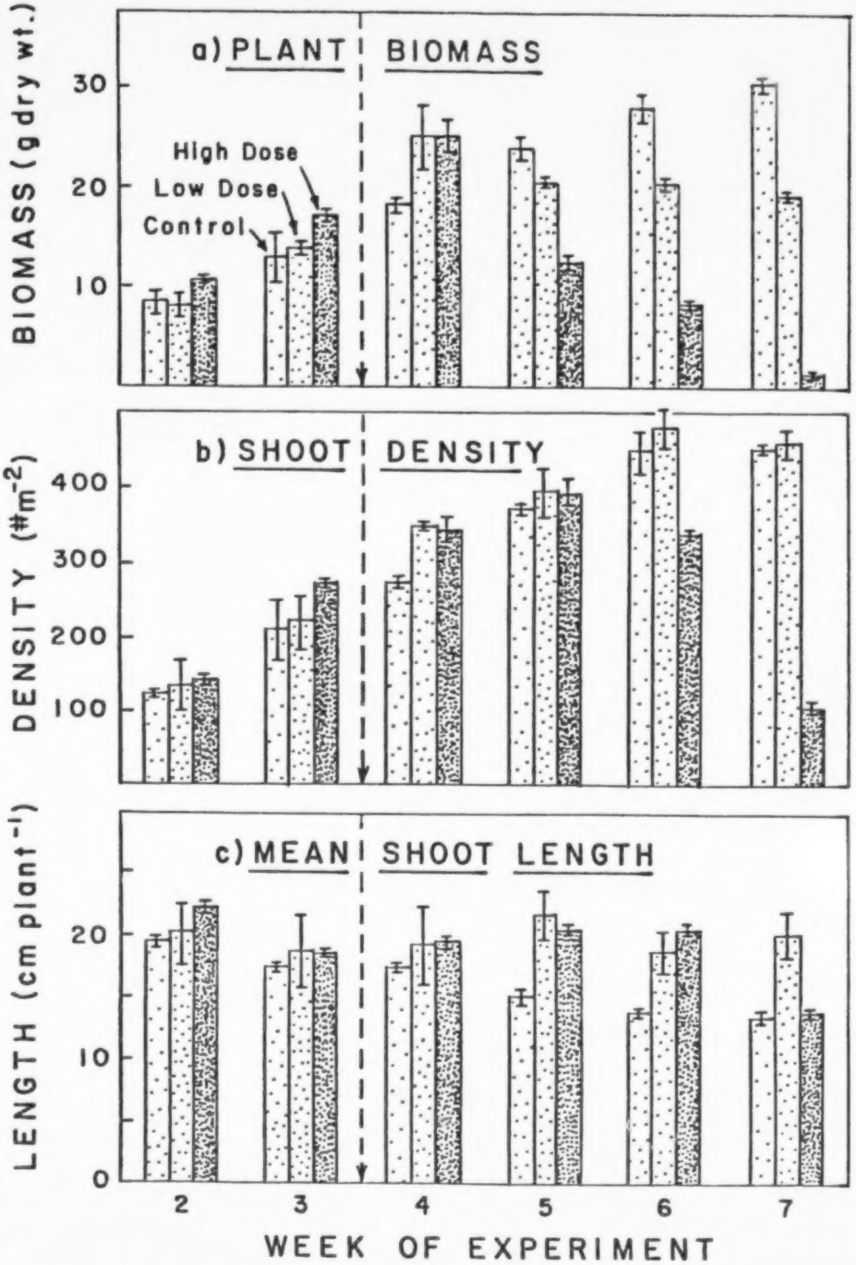

Fig. 3. Temporal responses of a) plant biomass, b) shoot density, and c) mean shoot length for Potamogeton perfoliatus exposed to low (0.13 ppm) and high (1.20 ppm) doses of atrazine compared to controls. Vertical bar indicate ± standard error, and dashed lines with arrows represents the initiation of treatment.

TABLE 2. Summary of selected physiological and morphological characteristics of Potamogeton perfoliatus populations in microcosm communities treated with the herbicide, atrazine.*

Measurement	Treatment		
	Control	Low	High
Shoot Biomass (B_a) (g d.w. m^{-2})	44.3 ± 17.1	24.3 ± 8.7	1.4 ± 0.1
Rhizobial Biomass (B_b) (g d.w. m^{-2})	40.0 ± 12.9	20.0 ± 8.6	-†
Ratio, B_b:B_a	0.93 ± 0.22	0.94 ± 0.54	-†
Shoot Density (no. m^{-2})	458 ± 13	477 ± 41	107 ± 6
Mean length of Shoots (cm $plant^{-1}$)	15.5 ± 2.9	20.2 ± 2.5	19.6 ± 1.9
Length:Weight Ratio** (m g^{-1})	2.4 ± 0.3	4.3 ± 0.6	6.1 ± 2.3
Chlorophyll a (mg m^{-2}(leaf))	28 ± 8	158 ± 16	114 ± 5
Internodal Length (cm)	1.37 ± 0.06	1.11 ± 0.09	1.89 ± 0.82

*Data are from samples taken in last week of experiment, except in the case of Shoot Lengths which are averaged over the last 3 wk. Given are mean values ± standard deviation, where n = 12 for Chlorophyll, n = 6 for Biomass and Shoot Length, and n=20-30 for Length:Weight and Internodal Length.

**Ratio for individual shoots from weeks 4, 7 and 8.

†Rhizobial biomass is not reported for high dose systems because plant material appeared to be dead and decaying.

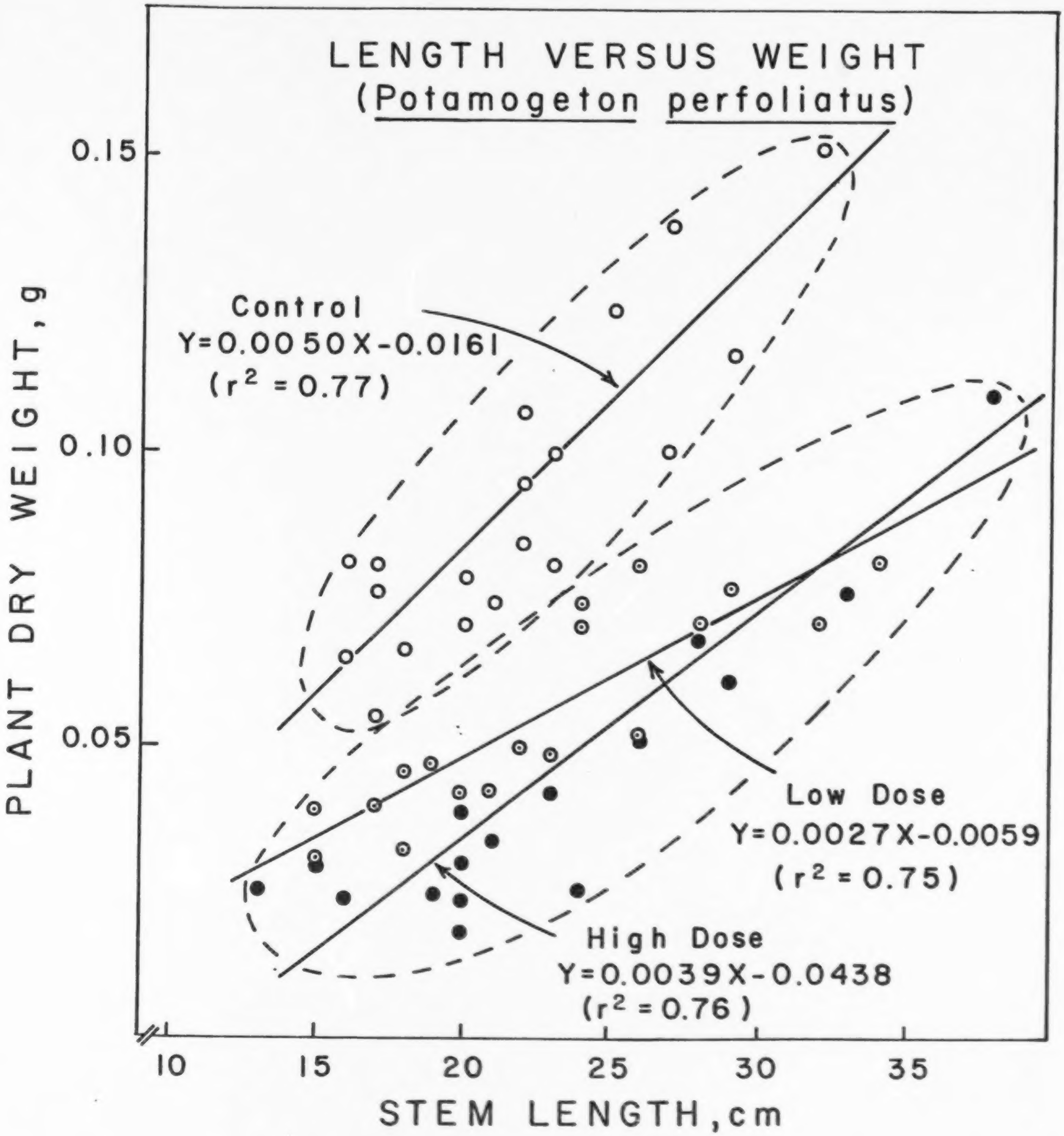

Fig. 4. Dry weight of plant stems versus stem length for Potamogeton perfoliatus in microcosms treated with atrazine at low (solid circles, 0.13 ppm) and high (circles with dots, 1.20 ppm) doses and for untreated plants in control (open circle) microcosms. Plant samples taken 2 and 4 wk after initial treatment.

consequence of negative values for P_a over the previous 4 wk. This 4 wk lapse between full metabolic and biomass response is consistent with the respiratory turn-over time (τ = biomass:respiration) just prior to treatment, where biomass was at about 15 g m^{-2} and respiration was 0.4 g $m^{-2}d^{-1}$ (τ = 37 d). Plant biomass in low dose microcosms remained constant after week 5 at low dose, suggesting again a recovery of metabolic balance (c.f., Fig. 2). While both herbicide dosages caused significant reduction in total standing stocks, the ratio of below-ground to above-ground biomass was unaffected by atrazine treatment (Table 2). Other investigators (Brooker and Edwards, 1973; Strange, 1976) have reported marked losses of macrophytic biomass after prolonged (30 d) exposures to high concentrations of herbicides (>0.5 ppm); however, the temporal correspondence between reductions in P_a and biomass have not been examined previously.

The size-distribution of P. perfoliatus shoots was unaffected by herbicide treatment in our experiments, with plants in the 6-10 cm class dominating throughout the experiment at all atrazine levels (Cunningham, 1979). Average length of individual shoots, however, was significantly increased for both low and high treatments within 2 wk following atrazine amendments (Fig. 3c, Table 2). In contrast, Forney and Davis (1981) reported the opposite response, with 26-51% reduction in growth of shoots for P. perfoliatus exposed to 0.1-0.32 ppm atrazine for 4 wk. This difference may be due to the relatively low light intensities (75 μEin $m^{-2}s^{-1}$) under which their experiments were conducted. The elongation effect which we observed is consistent with the change in length:weight ratio discussed above.

There is some evidence that the photosynthetic stress imposed by atrazine could elicit this increase in stem length, where for instance, Young and Evans (1978) found that leaf elongation and etiolation were an indication of atrazine stress and tolerance in perennial grasses. Stem and leaf elongation have been suggested by numerous investigators as indices of shade adaptation in submerged vascular plants. Spence (1975) has reviewed the substantial literature documenting this effect for various Potamogetonaceae under reduced intensity and quality of light in field and laboratory experiments. While the control mechanism for this response of submerged aquatic plants to decreased light is uncertain, it is of physiological interest that atrazine exposure produced a similar effect.

Another index of shade response by Potamogeton spp. and other SAV species with distinct leaf and stem structure (Spence 1975) is "internodal length" (i.e., the mean distance along the stem between leaf nodes). For atrazine treated P. perfoliatus, the effect on internodal length was less clear than for mean stem length and for L:B ratio. Although a significant (38%) increase in this index was evident for high dose plants compared to controls, there was a slight decrease for low atrazine treated plants. No pre-treatment differences were evident among microcosm plant populations. For this analysis, special effort was made to select plants representative of size distributions of respective experimental populations, since a significant relationship was found between internodal length (I, cm) and total stem length (L, cm),

$$I = 0.025\ L + 1.002 \qquad (r^2 = 0.83).$$

Thus, this index (internodal length) is subject to the vagueries of visually determined "representative samples".

Concentrations of chlorophyll a in P. perfoliatus leaves also exhibited a reaction to atrazine treatment which is analogous to that expected under extreme light limitation, where significantly elevated levels of chlorophyll a were measured for herbicide treated plants (Table 2). An inverse relationship between ambient light and chlorophyll a has been previously reported for various submerged plants (e.g., Bowes et al., 1977; Wiginton and McMillan, 1979; Drew, 1979).

Several investigators have observed this "greening effect" of increased chlorophyll content under atrazine stress for algae and terrestrial plants; however, we have found no previous reference to effects of atrazine on pigment composition in aquatic vascular plants (Ebert and Dumford, 1976). The physiological mechanisms whereby these reactions (elongation and increased chlorophyll) to atrazine stress might be effected are presently unclear (Ebert and Dumford, 1976; Moreland, 1980), as is the relationship (if any) to modes of shade adaptation.

Community Photosynthesis and Respiration

Apparent production (P_a), night respiration (R_n) and the metabolic ratio ($P_a:R_n$) for the integrated microcosm communities are provided in Fig. 5. The general response of these metabolic variables was similar to that for P. perfoliatus alone (Fig. 2), with significant effects on P_a at low and high dose and recovery of low dose P_a toward the end of the experiment. A distinct difference, however, was the pronounced decrease in community P_a following treatment, even for control microcosms. This may be partially attributable to the addition of sediments as a vehicle control; however, seston levels in post-treatment weeks actually decreased for all microcosms steadily over the course of the experiment (Table 1 and Cunningham, 1979). Probably, a more reasonable explanation lies in the sharp reduction in dissolved nutrients and associated phytoplankton populations. In control microcosms by week 4, concentrations of NH_4^+, NO_3^- and $PO^=_4$ decreased to 0.10, 0.15 and 0.14 μM respectively, and between weeks 3 and 4 algal P_a decreased by more than 50%. However, without untreated controls this remains an open question.

The response of community R_n to treatment (Fig. 5b) was almost identical to that for P. perfoliatus respiration, with significant effects evident only at high dosage. Community $P_a:R_n$ ratios also followed similar pattern to those for SAV (Fig. 5c), although ratios were generally below 1.0 (even for controls) indicating a net consumption of previously stored (probably sediment) detrital organic matter (Odum, 1956). Presumably, the lack of a strong response in community R_n to herbicide treatment may further reflect the relative importance of detrital (rather than direct grazing) trophic chains in these microcosms, since the loss of P_a did not produce a comparable reduction in R_n.

Few studies have reported effects of atrazine treatment on community P_a

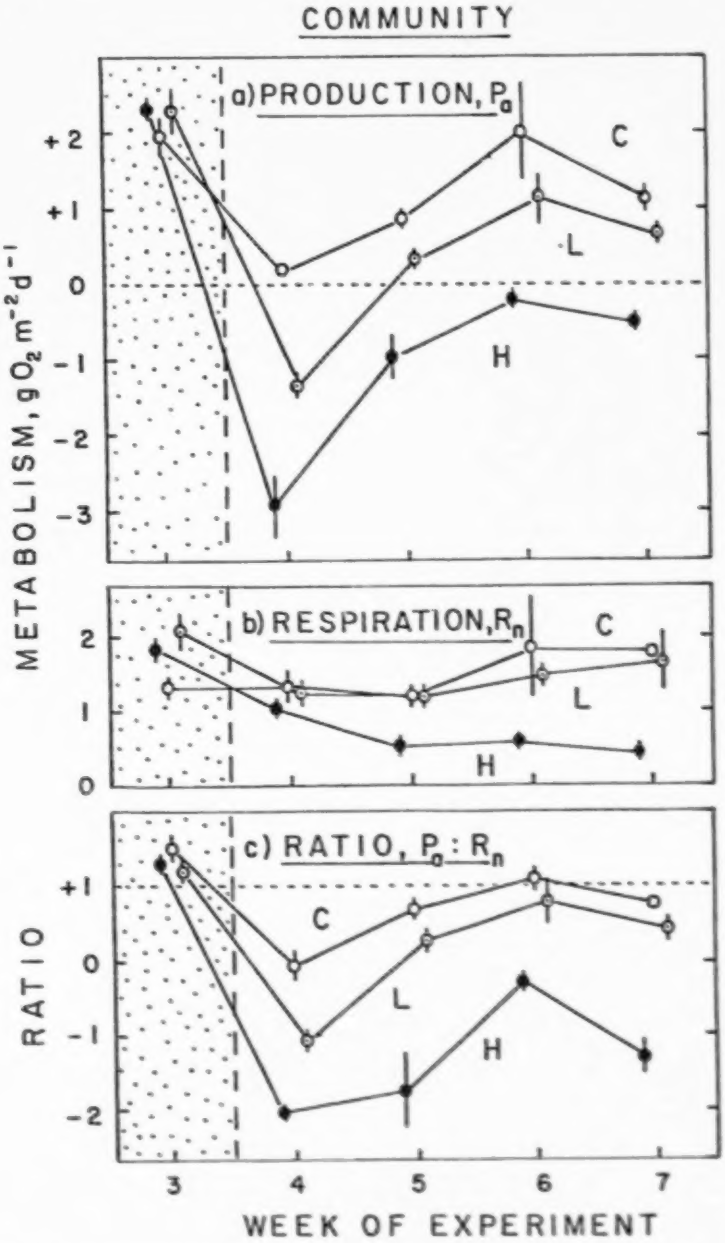

Fig. 5. Metabolic responses of microcosm communities containing Potamogeton perfoliatus to low (L = 0.13 ppm) and high (H = 1.20 ppm) dosages of atrazine as compared to controls (C), in terms of a) apparent O_2 production (P_a), b) night respiration (R_n), and c) the ratio P_a:R_n. Points and vertical bars indicate mean ± range and shaded portion of figure represents pretreatment period.

and R_n. However, two investigations have measured effects of other herbicides on community oxygen metabolism. Brooker and Edwards (1973 a,b) found that community metabolism in a reservoir treated with paraquat to control the SAV, Myriophyllum sp., exhibited an initial loss of P_a, followed by a return to pre-treatment levels within 1-2 wk. However, most of this recovery was attributed to increased phytoplankton production, with an eventual recolonization after 1-2 mo by macrophytic algae (Chara globularis) rather than SAV. Strange (1976) reported virtual loss of community P_a (as CO_2 uptake) in microcosms containing the SAV, Egeria densa, within 30 d following treatment with 0.5 ppm mixed herbicides (diquat and endothall). Daily measurements of P_a indicated little or no recovery after treatment. Here as in our study, the effect on respiration was less marked, so that P_a:R_n followed a pattern similar to that for P_a. In contrast, Brooker and Edwards (1973 a,b) observed losses of P_a and R_n to be similar, with little effect on P_a:R_n, probably because of the mechanism of paraquat action involving formation of generally reactive free-radicals.

We had hypothesized that the relative balance among major autotrophic groups (SAV, epiphytes, phytoplankton, and benthic microalgae) would shift in response to differential effects of herbicide treatment and resulting changes in competitive advantage for light and nutrients. However, our observations failed to corroborate this hypothesis. The relative contributions of these 4 groups to total gross O_2 production (operationally defined as P_a plus R_n) are given in Fig. 6. In control and low dose microcosms P. perfoliatus contributions increased steadily from 41 and 44% in week 3 to 77 and 74% in week 7, while the phytoplankton proportions decreased from 41 and 43% to 8 and 7% in control and low dose, respectively. Phytoplankton and epiphytes did comprise a slightly greater portion of total production under high dosage; however, the rates were all so low that it is hard to ascribe any particular importance to this. The absence of herbicide induced changes in the balance among autotrophs is probably the consequence of two major factors. First, herbicide toxicity may be similar for all photosynthetic organisms in the microcosm communities. Secondly, the particularly low concentrations of dissolved nutrients in microcosm water columns may have conferred overwhelming advantage to P. perfoliatus which was able to draw on higher concentrations available in sediment porewaters.

Final Comments

This experiment has demonstrated the ability of a submerged vascular plant, P. perfoliatus, to recover photosynthetic O_2 production within several weeks following continuous treatment with moderately high concentrations of the herbicide atrazine. Such observations emphasize the importance of examining temporal patterns over weeks or months for understanding the effects of herbicides on SAV. More subtle manifestations of effects such as changes in reproductive success would require even longer term experiments. We report here several other indices of atrazine effects such as stem elongation, reduced stem weight per unit length, increased internodal length and increased levels of chlorophyll a, all of which are parallel to the previously observed response of this and related species to reduced light intensities. Such indices could be used routinely as measures of atrazine stress; however, care must be taken not to confuse plant responses to herbicide and shade. The effects of atrazine

TABLE 3. Coefficients of variation (CV) for apparent production in microcosm communities treated with the herbicide, atrazine.*

Treatment Component	Week of Experiment				
	3†	4	5	6	7
CONTROL					
Macrophyte	40.2†	21.3	20.8	40.5	17.2
Plankton	37.8†	12.4	65.4	64.3	95.4
LOW DOSE					
Macrophyte	58.6†	50.3	38.3	23.4	40.2
Plankton	27.0†	34.2	122.8	64.3	111.9
HIGH DOSE					
Macrophyte	26.3†	174.4	115.6	136.1	80.0
Plankton	19.3†	171.4	85.3	137.1	166.7

*CV is defined as (standard deviation/mean) x 100.
†Week 3 is prior to treatment; weeks 4-7 are post-treatment.

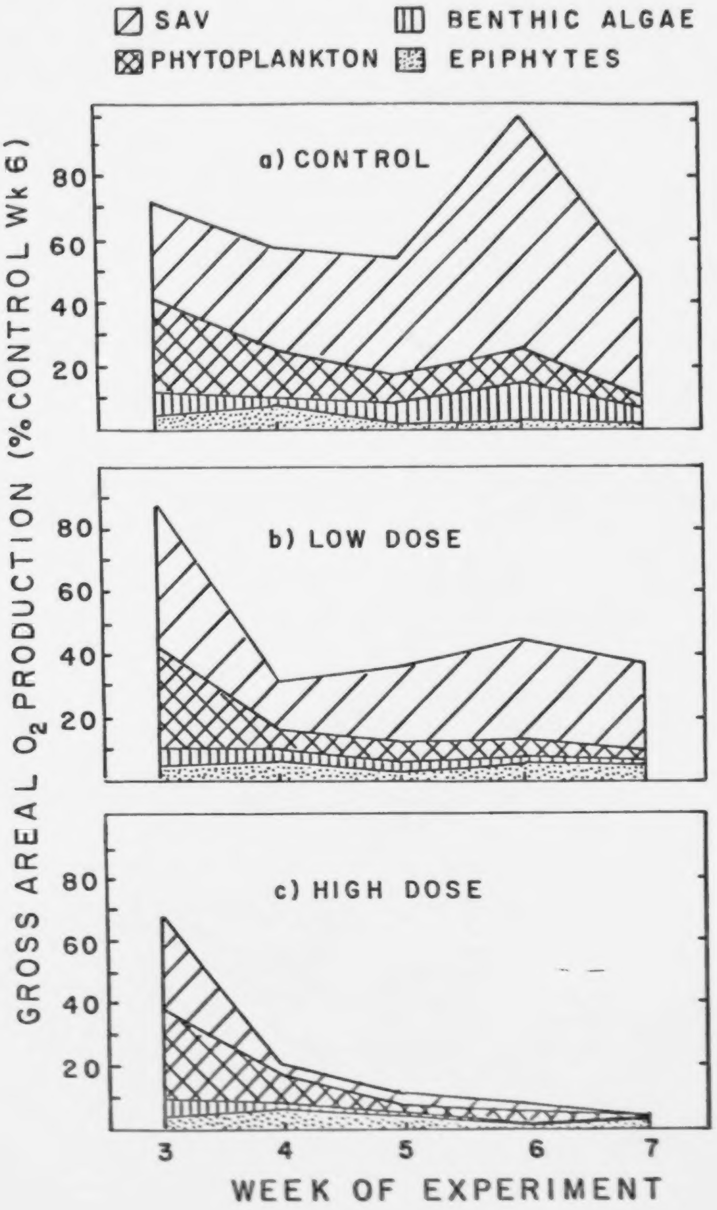

Fig. 6. Relative contributions (compared to control microcosm in week 6) of Potamogeton perfoliatus (SAV), phytoplankton, benthic microalgae and epiphytic algae to total gross O_2 production (defined as P_a plus R_n) of experimental communities in a) control, b) low (0.13 ppm) atrazine dosed, and c) high (1.20 ppm) atrazine dosed microcosms. Treatment was initiated 1 d prior to week 4 measurements.

on competitive interactions among primary producers in SAV communities may not involve compensatory shifts in relative balance. However, this question needs to be addressed in experimental systems where both sediment and aqueous nutrients are continuously available. By characterizing a broad range of atrazine effects on *P. perfoliatus* physiology and morphology, this study has provided a basis for further examination of SAV responses to herbicides at lower concentrations typical of those occurring frequently in estuaries such as Chesapeake Bay.

REFERENCES

1. Adams, S.M. (1976). The ecology of eelgrass, Zostera marina (L.), fish communities. I. Structural analysis. J. exp. mar. Biol. Ecol. 22:269-291.

2. Bayley, S., Stotts, V.D., Springer, P.F., Steenis, J. (1978). Changes in submerged aquatic macrophyte populations at the head of the Chesapeake Bay, 1958-1975. Estuaries 1:74-75.

3. Bielecki, K., Skrabka, H. (1976). Effect of some herbicides on photosynthesis of Spirodela polyrhiza (Lemnaceae). Acta Agrobot. 29: 59-68.

4. Bowes, G., Van, T.K., Garrard, L.A., Haller, W.T. (1977). Adaptation to low light levels by Hydrilla. J. Aquat. Plant Mgmt. 15:32-35.

5. Brooker, M.P., Edwards, R.W. (1973a). Effects of the herbicide paraquat on the ecology of a reservoir. I. Botanical and chemical aspects. Freshwat. Biol. 3:157-75.

6. Brooker, M.P., Edwards, R.W. (1973b). Effects of the herbicide paraquat on ecology of a reservoir. II. Community metabolism. Freshwat. Biol. 3:383-89.

7. Brooker, M.P., Edwards, R.W. (1975). Aquatic herbicides and the control of water weeds. Water Res. 9:1-15.

8. Buesa, R.J. (1975). Population biomass and metabolic rates of marine angiosperms on the northwestern Cuban shelf. Aquatic Bot. 1:11-23.

9. Coen, L.D., Heck, K.L., Abele, L.G. (1981). Experiments on competition and predation among shrimps of seagrass meadows. Ecology 62:1484-1493.

10. Conover, J.T. (1968). The importance of natural diffusion gradients in the metabolism of benthic marine plants. Botanica Mar. 11:1-9.

11. Correll, D.L., Wu, T.L. (1982). Atrazine toxicity to submersed vascular plants in simulated estuarine microcosms. Aquatic Bot. (In press).

12. Cunningham, J.J. (1979). Responses of microcosm communities containing submerged aquatic vegetation to herbicide stress. MS Thesis, Univ. of Maryland, College Park. 66 pp.

13. Den Hartog, C., Polderman, P.J.G. (1975). Changes in the seagrass populations of the Dutch Waddenzee. Aquatic Bot. 1:141-147.

14. Drew, E.A. (1979). Physiological aspects of primary production in seagrasses. Aquatic Bot. 7:139-50.

15. Ebert, E., Dumford, S.W. (1976). Effects of triazine herbicides on the physiology of plants. Residue Reviews 65:1-103.

16. Fonseca, M.S., Fisher, J.S., Zieman, J.C., Thayer, G.W. (1982). Influence of the seagrass, Zostera marina L., on current flow. Estuar. Coast. Shelf Sci. 15:351- 364.

17. Forney, D.R., Davis, D.E. (1981). Effects of low concentrations of herbicides on submersed aquatic plants. Weed Sci. 29:677-85.

18. Fowler, M.C. (1977). Laboratory trials of a new triazine herbicide (DPX 3674) on various aquatic species of macrophytes and algae. Weed Res. 17, 191-195.

19. Funderbunk, H.H., Lawrence, J.M. (1963). Absorption and translocation of radioactive herbicides in submersed and emersed aquatic weeds. Weed Res. 3:304-11.

20. Ginsburg, R.N., Lowenstam, H.A. (1958). The influence of marine bottom communities on the depositional environment of sediment. J. Geol. 66: 310-318.

21. Goldsborough, W.J. 1983. Light-shade adaptation for the submerged vascular plant, Potamogeton perfoliatus. MS Thesis, Univ. Maryland, College Park (In press).

22. Grasshoff, K., Johannsen, H. (1972). A new sensitive and direct method for the automatic determination of ammonia in seawater. J. Cons. lut. Explor. Mer. 34:516-521.

23. Harlin, M.M., Thorne-Miller, B., Boothroyd, J.C. (1982). Seagrass-sediment dynamics of a flood-tidal delta in Rhode Island (USA). Aquatic Bot. 14:127-138.

24. Howard-Williams, C. (1981). Studies on the ability of a Potamogeton pectinatus community to remove dissolved nitrogen and phosphorus compounds from lake water. J. Appl. Ecol. 18:619-637.

25. Iizumi, H., Hattori, A., McRoy, C.P. (1980). Nitrate and nitrite in the interstitial waters of eelgrass beds in relation to the rhizosphere. J. exp. mar. Biol. Ecol. 47:191-201.

26. Jones, T.W., Kemp, W.M., Stevenson, J.C., Means, J.C. (1982). Degradation of atrazine in estuarine water/sediment systems and selected soils. J. Environ. Qual. (In press).

27. Jupp, B.P., Spence, D.H.N. (1977). Limitations on macrophytes in a eutrophic lake, Loch Leven. I. Effects of phytoplankton. J. Ecol. 65:175-186.

28. Kemp, W.M., Lewis, M.R., Cunningham, J.J., Stevenson, J.C., Boynton, W.R. (1981). Microcosms, macrophytes, and hierarchies: Environmental research in the Chesapeake Bay. Microcosm Research in Ecology. (Ed. by J. Giesy). ERDA Conf. Ser., NTIS, Springfield, Virginia. 911-36.

29. Kemp, W.M., Means, J.C., Jones, T.W., Stevenson, J.C. (1982). Herbicides in Chesapeake Bay and their effects on submerged aquatic vegetation. Univ. Maryland Centr Environ. Est. Stud. Ref. No. 1295-HPEL. Horn Pt. Env. Labs, Cambridge, MD. 82 pp.

30. Kenworthy, W.J., Zieman, J.C., Thayer, G.W. (1982). Evidence for the influence of seagrasses on the benthic nitrogen cycle in a coastal plain estuary near Beaufort, North Carolina (USA) Oecologia 54:152-158.

31. Kiorboe, T. (1980). Distribution and production of submerged macrophytes in Tipper Grund (Ringkobing Fjord, Denmark), and the impact of waterfowl-grazing. J. Appl. Ecol. 17:657-687.

32. Kullberg, R.G. (1974). Distribution of aquatic macrophytes related to paper mill effluents in a Southern Michigan stream. Amer. Midl. Nat. 91:271-281.

33. Lewis, M.R. (1980). An investigation of some homostatic properties of model ecosystems in terms of community metabolism and component interactions. MS Thesis, Univ. of Maryland, College Park. 150 pp.

34. Lewis, M.R., Kemp, W.M., Cunningham, J.J., Stevenson, J.C. (1982). A rapid technique for preparation of aquatic macrophyte samples for measurin ^{14}C incorporation. Aquatic Bot. (In press).

35. Mann, K.H. (1982). Ecology of coastal waters: a system approach. Univ. of California Press, Berkeley.

36. Marsh, A.G. (1973). The *Zostera* epifaunal community in the York River, Virginia. Chesapeake Sci. 14:87-97.

37. McRoy, C.P., Barsdate, R.J., Nebert, M. (1972). Phosphorus cycling in an eelgrass (*Zostera marina* L.) ecosystem. Limnol. Oceanogr. 17:58-67.

38. Moreland, D.E. (1980). Mechanisms of action of herbicides. Ann. Rev. Plant Physiol. 31:597-638.

39. Nagel, J.S. (1968). Distribution of the epibiota of macrobenthic plants. Contr. Mar. Sci., Univ. Texas. 13:105-144.

40. Negi, N.S., Funderbunk, H.H., Davis, D.E. (1964). Metabolism of atrazine by susceptible and resistant plants. Weeds 12:52-57.

41. Nelson, W.G. (1979). An analysis of structural pattern in an eelgrass (*Zostera marina* L.). amphipod community. J. exp. mar. Biol. Ecol. 39:231-264.

42. Odum, E.P. (1971). Fundamentals of Ecology. W.B. Saunders, Philadelphia. 574 pp.

43. Odum, H.T. (1956). Primary production in flowing waters. Limnol. Oceanogr. 1:102-17.

44. Odum, H.T., Hoskin, C.M. (1958). Comparative studies on the metabolism of marine waters. Publ. Inst. Mar. Sci. (Texas) 8:23-55.

45. Orth, R.J. (1975). Destruction of eelgrass, *Zostera marina*, by the cownose ray *Rhinoptera bonasus*, in the Chesapeake Bay. Chesapeake Sci. 16:205-208.

46. Orth, R.J., Moore, K.A. (1981). Submerged aquatic vegetation of the Chesapeake Bay: Past, present and future. Trans. N. Amer. Wildl. Nat. Res. Conf. 46, 271-83.

47. Peres, J., Picard, J. (1975). Causes de la raréfaction et de la disparition des herbiers de *Posidonia oceanica* sur les cotes Francaises de La Mediterranée. Aquatic Bot. 1:133-39.

48. Phillips, G.L., Eminson, D.F., Moss, B. 1978. A mechanism to account for macrophyte decline in progressively enriched freshwaters. Aquatic. Bot. 4:103-126.

49. Purkayastha, R., Cochrane, W.P. (1973). A method for analysis of atrazine. J. Agric. Food Chem. 2:93-102.

50. Robson, T.O., Barrett, P.R.F. (1977). Review of effects of aquatic herbicides. In Perring, F.H., Mellanby, K. (eds.). Ecological Effects of Pesticides. Linnean Soc. Symp. Ser., No. 5, Academic Press, New York, pp. 111-31.

51. Sand-Jensen, K., Søndergaard, M. (1981). Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. Int. Rev. ges. Hydrobiol. 66:529-552.

52. Snedecor, G.W., Cochran, W.G. (1967). Statistical Methods. Iowa State Univ. Press. Ames.

53. Souza-Machado, V., Arntzen, C.J., Bandeen, J.D., Stephenson, G.R. (1978). Comparative triazine effects upon system II photo-chemistry in chloroplasts of two common lambsquarters (*Chenopodium album*) biotypes. Weed Sci. 26:318-22.

54. Spence, D.H.N. (1975). Light and plant response in freshwater. In: Evans, G.C., Bainbridge, R., Rockham (eds.). Light as an Ecological Factor. Blackwell Sci. Publ., Oxford, pp. 93-133.

55. Strange, R.J. (1976). Nutrient release and community metabolism following application of herbicide to macrophytes in microcosms. J. Appl. Ecolo. 13:889-897.

56. Strange, R.M., Schreck, C.B. (1976). Response of aerobic community metabolism to chemical treatment of aquatic macrophytes. J. Aquatic Plant Mange. 14:45-50.

57. Strickland, J.D.H., Parsons, T.R. (1972). A Practical Handbook of Seawater Analysis. Fish Res. Bd., Canada Bull. 167. Ottawa. 310 pp.

58. Sutton, D.C., Durham, D.A., Bingham, S.W., Foy, C.L. (1969). Influence of simazine on apparent photosynthesis of aquatic plants and herbicide residue removal from water. Weed Sci. 17:56-59.

59. Vernon, L.P. (1960). Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Anal. Chem. 32:1144-50.

60. Westlake, D.F. (1963). Comparisons of plant productivity. Biol. Rev. 38:385-425.

61. Westlake, D.F. (1978). Rapid exchange of oxygen between plant and water. Verh. Intern. Verein. Limnol. 20:2363-67.

62. Wiginton, J.R., McMillan, C. (1979). Cholorophyll composition under controlled light conditions as related to the distribution of seagrasses in Texas and the U.S. Virgin Islands. Aquatic Bot. 6:171-84.

63. Young, J.A., Evans, R.A. (1978). Etiolated growth of range grasses for an indication of tolerance to atrazine. Weed Sci. 26:480-83.

64. Zieman, J.C., Wetzel, R.G. (1980). Productivity in Seagrasses: Methods and Rates. In: Phillips, R.C., McRoy, C.P. (eds.). Handbook of Seagrass Biology: An Ecosystem Perspective. Garland STPM Press, New York, pp. 87-118.

CHAPTER III

RESPONSE OF *POTAMOGETON PERFOLIATUS* AND *MYRIOPHYLLUM SPICATUM* PHOTOSYNTHESIS AND GROWTH TO ATRAZINE AND LINURON STRESS IN ESTUARINE MICROCOSMS*

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INTRODUCTION

The littoral waters of Chesapeake Bay historically have been dominated by productive communities of submerged vascular plants (e.g. Bayley et al. 1978; Stevenson and Confer 1978). This submerged aquatic vegetation (SAV) can provide food, habitat and refuge from predators for many fish and invertebrates during crucial stages of their growth cycle (Adams 1976). Moreover, SAV communities stabilize surrounding estuarine ecosystems by trapping and binding sediments (Orth 1977), and by modulating pulses in estuarine nutrient cycles (Barsdate et al. 1974; Howard-Williams 1981).

Over the last two decades, the numerous species of submerged macrophytes native to Chesapeake Bay have undergone a dramatic decline in abundance and distribution, so that current populations in the upper and middle bay are less than 5% of their previous levels (Stevenson and Confer 1978; Orth and Moore 1981). Just prior to this reduction of native SAV species, there was an unprecedented invasion of the exotic SAV, Myriophyllum spicatum, which grew to nuisance proportions in a period of 3-5 years, after which it too decreased rapidly (Bayley et al. 1978).

The cause of this decline has been a subject of considerable debate over the last several years; however, one of the primary factors postulated as a causative agent is the runoff of agricultural herbicides (Kemp et al. 1982). The vast agricultural enterprise in Chesapeake Bay's watershed employs a variety of phytotoxic compounds for weed control. Two of the most widely used herbicides in this region are atrazine and linuron. In some previous experiments the phytotoxic effects of atrazine were tested for the macrophyte, Potamogeton perfoliatus (Cunningham 1979). This plant, which is one of the dominant native species in middle and upper Chesapeake Bay, exhibited strong photosynthetic inhibition at herbicide levels of about 100 and 1000 ppb. However, even the lower of these conccentrations is slightly greater than the highest levels measured in runoff water entering the estuary (Hershner et al. 1982). Other recent studies have reported variable results regarding growth and mortality responses of this genus to various concentrations of atrazine (Forney and Davis 1981; Correll and Wu 1982), although some supression of growth was generally evident at 10-30 ppb (Forney and Davis 1981).

The purpose of the present study was to further investigate herbicide phytotoxicity in the esturine environment, examining additional compounds and plant species over a broader range of herbicide concentrations. In addition to P. perfoliatus we selected M. spicatum for these experiments to test the hypothesis that it is more tolerant to herbicide stress than the native species, thus possibly explaining its invasion just prior to onset of the general SAV decline. Experiments were conducted using two herbicides, atrazine and linuron, each of which is considered representative of a whole class of compounds (Kemp et al. 1982). The study design employed nominal herbicide concentrations of 0, 5, 50, 100, 500 and 1000 ppb aqueous, and plant response was measured in terms of apparent production and respiration as well as growth and abundance. Herbicide concentrations in these experiments were compared to those observed in an extensive estuarine monitoring program to interpret these experimental results in terms of the potential role of herbicides in the SAV decline.

MATERIALS AND METHODS

Experimental Systems

Experimental microcosm communities were established in 42 glass aquaria (32x 36 x 48 cm), each containing about 50 ℓ of filtered (5 μ) estuarine water and about 8 cm depth of sediment. Microcosms were seeded with 10 individual *Potamogeton perfoliatus* or *Myriophyllum spicatum* plants of 6-14 cm stem length (2-6 cm rhizome) inserted into the sediments. Sediments were a sandy silt similar to that described in Cunningham (1979). Water, sediments and plants were all obtained from the brackish (8-12) ppt portion of the Choptank River estuary, a tributary of Chesapeake Bay.

Microcosms were maintained in an air-conditioned laboratory providing relatively constant water temperature (21.5 ± 1.5 C) throughout the study. Salinity was kept at 9.0 ± 1.1 ppt by replacing evaporative losses with distilled water. Illumination was provided by banks of cool white fluorescent lights on a 14 h photoperiod, yielding about 150-200 μEin $m^{-2}s^{-1}$ at the water surface. Water was continuously recirculated using centrifugal submerged pumps with inlet and outlet diffusers at opposite ends of each aquarium. The inner walls of these microcosms were scraped clean of epibota at weekly intervals.

Photosynthesis and Respiration

Apparent photosynthesis (P_a) and night respiration (R_n) for each plant community were estimated twice weekly on successive days using measurements of dissolved oxygen (DO) in the water column at dawn and dusk (Odum and Hoskins 1958; McConnell 1962). Concentrations of DO were maintained above 3-4 ppm throughout the study by periodic aeration with standard aquarium bubblers.

Corrections for gas transfer across the air-water interface were made by applying a diffusion coefficient to the observed mean departure from saturation (Odum and Hoskins 1958). Appropriate coefficients were obtained by measuring the rate of oxygen invasion into filtered (1.0 μm) aquarium water (previously deoxygenated by bubbling with N_2 gas). These aquaria contained no sediment, and a control with DO near saturation was utilized to account for any DO change due to microbial metabolism. In all, 5 such diffusion experiments were conducted and 2 examples are provided in Fig. 1. Coefficients ranged from 0.15-0.22 $gO_2\ m^{-2}h^{-1}\ atm^{-1}$ and averaged 0.18. Periodically, P_a and R_n were determined by measuring DO at 2-4 h intervals over an entire diel sequence, and these experiments demonstrated that the error in estimating metabolic

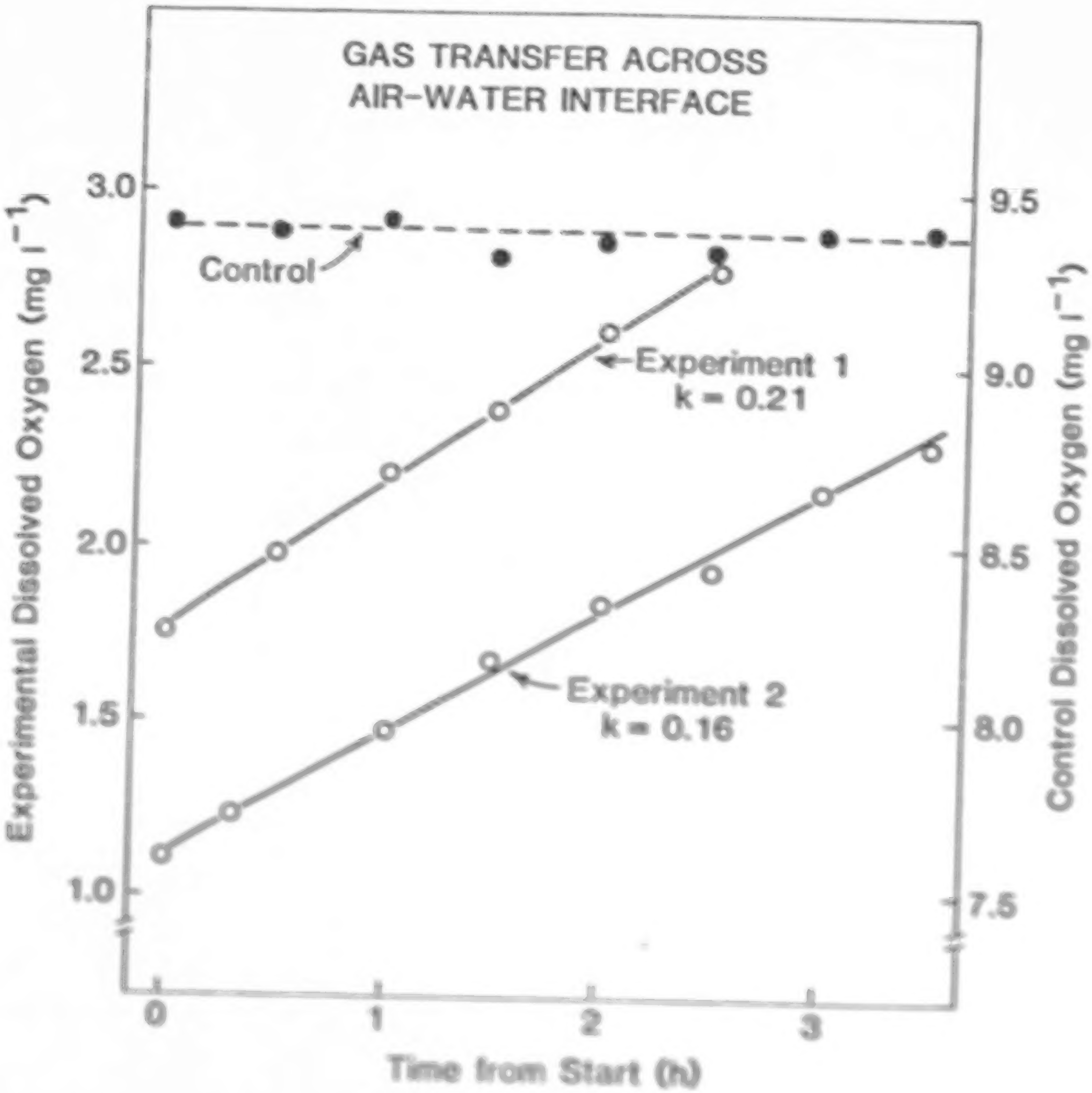

Figure 1. Example results of two experiments to measure the coefficient of O_2 diffusion across the air-water interface in experimental microcosms.

rates from DO at dawn and dusk only was small (<5%). Examples of 2 such diel patterns for control (Fig. 2a) and treated (Fig. 2b) microcosms with P. perfoliatus are presented in Fig. 2. Reaeration (diffusion) corrections were either positive (Fig. 2a) or negative (Fig. 2b) but were always <10-15% of P_a.

Contribution of plankton to the observed DO changes in microcosm water were estimated during the first experiment by incubating duplicate clear and opaque BOD bottles containing aquarium water. Again, P_a and R_n were inferred from DO changes in light and dark bottles which were incubated for 2-3 h suspended in front of the circulation pump outlet diffuser to gently and continually mix them. Plankton contribution to total water DO changes was generally neglibible (<5%), and therefore was not measured in the second experiment.

Plant Abundance and Biomass

Plant density (shoots/m^2) and shoot length were determined in each microcosm at 3-4 wk intervals for the atrazine experiment. At each sampling all stems were counted and measured for length. At the beginning and end of experiments several representative plants were selected from each microcosm, and these were measured individually and dried to constant weight at 60C (≥72h). Regressions of dry weight versus stem length were obtained and used to infer biomass from the stem density and length surveys. Initial biomass (i.e. weight of plants inserted in sediment) was estimated more directly by obtaining a blotted wet weight prior to planting and developing a regression of wet to dry weight on a subsample of 35-40 plants (Fig. 3). When experiments were terminated, all plants were harvested from each microcosm and shoots were dried at 60C to constant weight. Biomass of roots and rhizomes was not considered here, since it was found to be proportional to shoot biomass for all herbicide treatments in an earlier study (Cunningham 1979), and since it was ≤30% of total plant biomass in control microcosms (Fig. 4).

Herbicide Dosing, Sampling and Analysis

After establishing the microcosm communities, a period of 7-8 wk was allowed for development and equilibration prior to herbicide dosing. Technical grade (96.4% purity) atrazine was dissolved in 100 ml of methanol for delivery into the aquaria at nominal concentrations (calculated on the basis of microcosm water volume) of 0, 5, 50, 100, 500 and 1000 ppb, with both untreated and vehicle (methanol only) controls. Duplicate microcosms were randomly selected for each treatment.

At the termination of these experiments, samples of water, sediment and plant material were obtained from each aquarium for herbicide analysis. Two liters of water were collected in glass bottles and stored at 4C until analysis. Approximately 1.0 kg of sediment was collected from each system using brass corers, and these samples were frozen at -40C in glass bottles until analysis. Samples of plant material were drained, wrapped in aluminum foil and frozen at -40C until analysis. The extraction, clean-up and concentration procedures for atrazine and linuron were similar and, therefore, will be presented together. All glassware and apparatus used in the extraction and clean-up procedures were

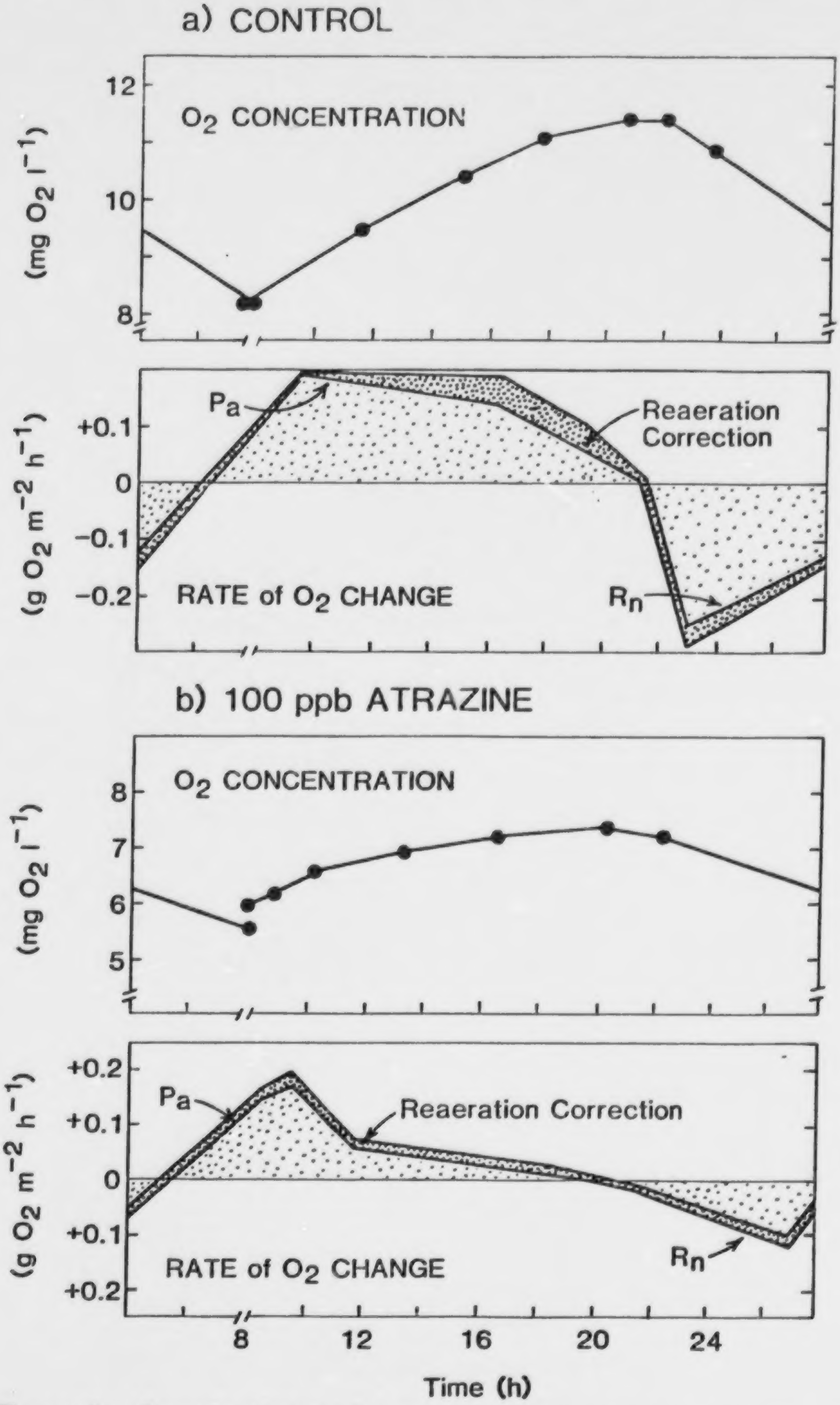

Figure 2. Example diel patterns of O_2 concentration and rate of change in O_2 with correction for gas exchange for a) control microcosm and b) 100 ppb atrazine treatment. Apparent O_2 production (P_a) and night respiration (R_n) are indicated.

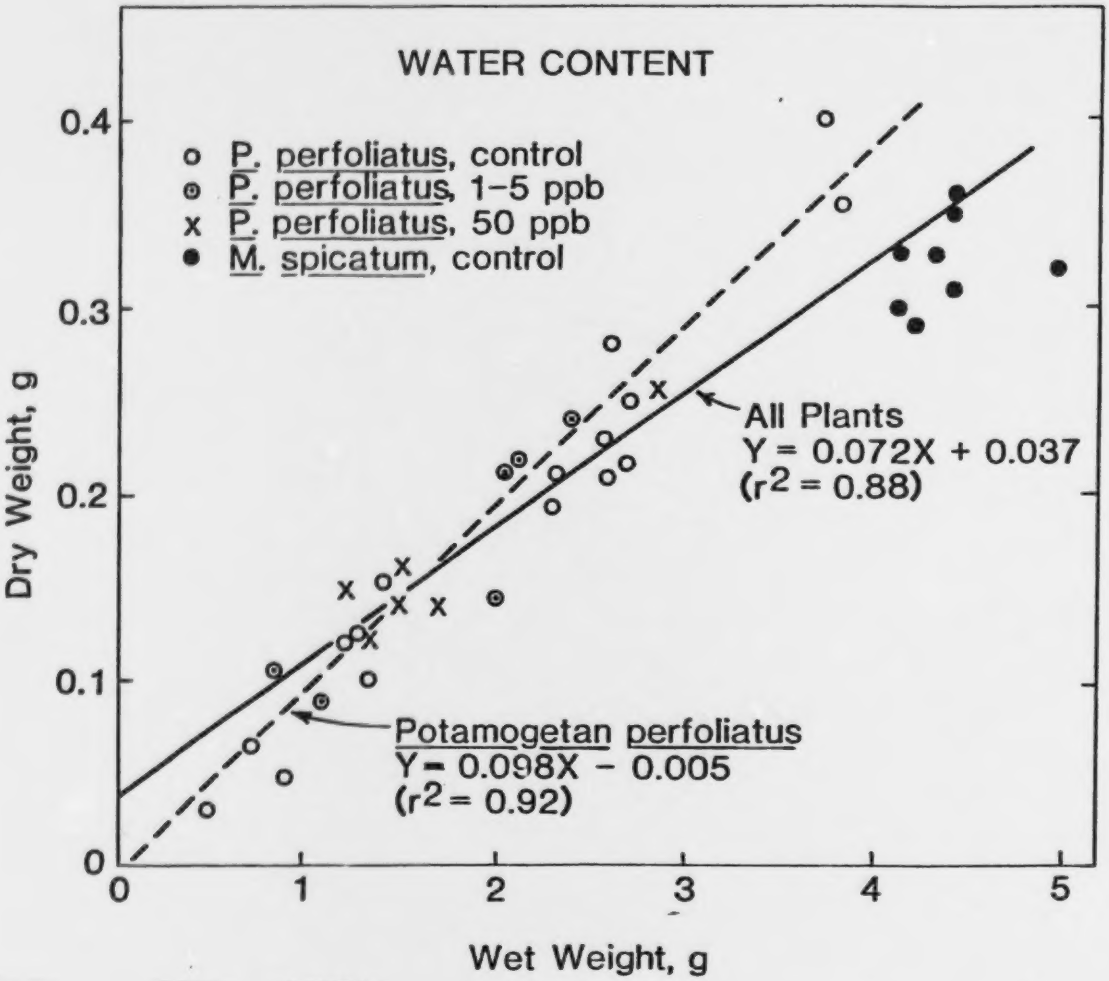

Figure 3. Water content (dry versus wet weight) for *Potamogeton perfoliatus* and *Myriophyllum spicatum* used in microcosm experiments.

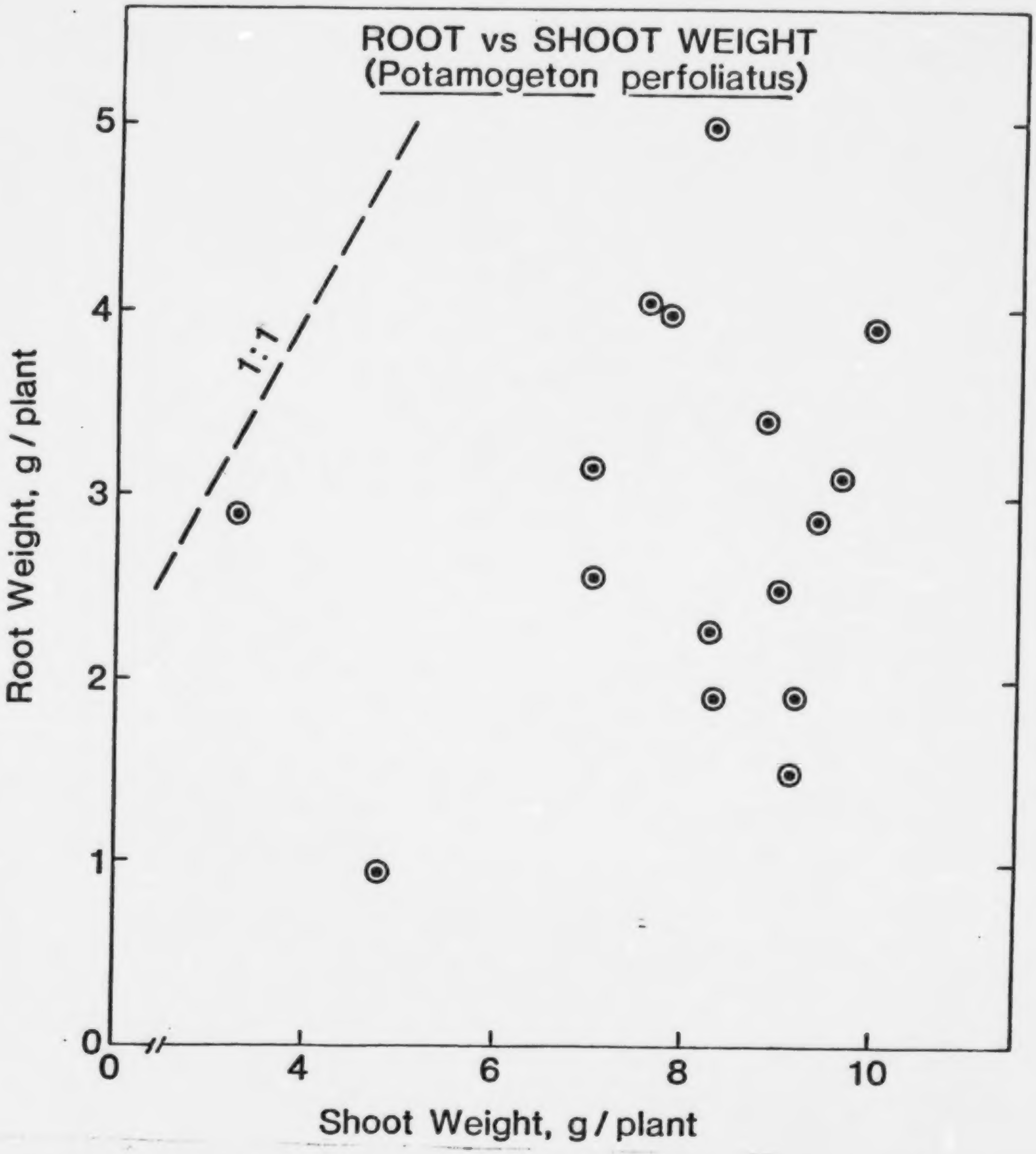

Figure 4. Belowground (Root) versus aboveground (Shoot) weights for *Potamogeton perfoliatus* used in microcosm experiments.

washed with glass distilled deionized water, and pesticide grade methanol prior to use.

Extraction of frozen sediment and plant tissue samples was achieved by weighing the frozen material in a tared, pre-extracted Soxhlet thimble. Samples were exhaustively extracted in a Soxhlet apparatus for 24 h with 200 ml of distilled-in-glass (DIG) methanol (Burdick and Jackson, Milwaukee, WI). Following extraction, sediment or tissue samples were oven-dried over night at 105C. Methanol extracts were dried over anhydrous sodium sulfate, concentrated in a rotary vacuum evaporator to approximately 10 ml, and taken to dryness under a stream of pure nitrogen. The residue was dissolved in 1 ml of DIG methanol for analysis. Some samples required clean-up prior to analysis, where the entire 1 ml sample was passed through a preparative reverse-phase liquid chromatographicc column (Bondapak C_{18}) using a solvent system of 50:50 methanol:water. The fraction(s) for atrazine and/or linuron were collected, evaporated under nitrogen and dissolved in 1 ml of DIG methanol for quantitative analysis.

Water samples (1-2 ℓ) in glass bottles were filtered through pre-combusted Whatman GF/C glass-fiber filters and then pumped at a rate of 3 ml/min through a SEP-PAK C_{18} (Waters Associates) cartridge which was pre-cleaned with 4 ml each of DIG acetonitrile, DIG methanol and distilled-deionized (DDI) water. After this procedure, the bottle was washed with 50 ml of DDI water, and this was also pumped through the SEP-PAK. Finally, 15 ml of DDI water was passed through the SEP-PAK to remove salt. The SEP-PAK was eluted with 5 ml of DIG methanol, and the eluent was evaporated under a stream of nitrogen and dissolved in 1 ml of DIG methanol containing a known quantity of an internal standard (diphenylamine) for quantitative analysis.

Quantitative analysis was performed using a combination of gas chromatography and liquid chromatography. Atrazine residues were quantified using a Hewlett-Packard 5840 gas chromatograph equipped with 2 m length of all-glass column (2 mm ID) packed with 10% Carbowax 20 M on 80/100 Supelcoport. The column was maintained at a temperature of 160C, andd injector and detector were held at 225C and 300C, respectively. An alkali-flame nitrogen-phosphorus specific detector was used to measure the amount of atrazine and internal standard in a 4 μl sample. Quantification was achieved by comparing the atrazine/internal standard ratio (AT/IS) to a calibration curve of AT/IS versus atrazine concentration.

Linuron and atrazine residues were measured using a Waters Associates 6000A liquid chromatograph equipped with a μ-Bondapak-Phenyl (T.M.) column, a model 440 fixed wavelength ultraviolet detector (254 μm), a Perkin-Elmer M-2 electronic integrator and a Linear 159 chart recorder. Samples (20 μℓ) were introduced into the column using a Rheodyne 7125 injection valve and eluted using an isocratic solvent system of 35% accetonitrile/water. Areas under the sample peaks were integrated and compared to a calibration curve for the compound.

Physical and Chemical Variables

Dissolved oxygen and temperature measurements were obtained using polarographic oxygen electrodes (Orbisphere Model 2709) calibrated daily in moist air. Salinity was measured at the beginning of each week using a Beckman (Model RS5-3) induction salinometer. Photosynthetically active radiation was determined weekly in selected microcosms at mid-water depth using a LICOR (Model-LI-192SB) cosine-corrected sensor. Concentrations of dissolved inorganic nitrogen species (NH_4^+, NO_2^- and NO_3^-) were measured on microcosm water passing a Whatman GFC (1.2 μ) filter using a Technicon Auto-Analyzer system. Nitrate and nitrite were determined according to the cadmium-reduction method, and ammonium analysis employed the Solarozano technique (Strickland and Parsons 1972).

Statistical Approach

Several statistical tests were utilized to identify significant effects of herbicides in the microcosm experiments. All analyses were done using apparent photosynthesis (P_a) and biomass as the variables indicating macrophyte performance. Testing of effects on P_a was divided into two stages involving pre-treatment and post-treatment data. In the first stage, pre-treatment data were subjected to an analysis of co-variance to determine if differences in the rate of increase of P_a among microcosms developed during the 4-5 wk period prior to herbicide treatment. In addition, the means of pre-treatment data were compared to determine if photosynthetic rates were equal in all treatments at the start of the experiment. This pre-treatment analysis was essential in the present study because differences among microcosms could develop in response to factors (such as sediment conditions) not related to herbicide treatment, and such differences would invalidate post-treatment comparisons among experimental systems. The second stage of our statistical interpretation consisted of one-way analysis of variance (ANOVA) testing, where herbicide concentration was the treatment. Tests were run independently for observations in each post-treatment week and significant differences among means were identified using the Student-Newman Kuels (SNK) multiple range test. Analysis of post-treatment data was done both with and without correction for pre-treatment differences in P_a. Biomass data were tested using simple one-way ANOVA with SNK.

RESULTS AND DISCUSSION

Atrazine Experiments

For the sake of clarity, atrazine and linuron experiments will be discussed separately. However, these will be compared and the overall implications considered in a final section.

Atrazine Concentrations

Atrazine concentrations remained relatively unchanged through the experimental period, with 84-89% of the original dose remaining after 4 wk (Table 1). Assuming first-order degradation kinetics, this is equivalent to a half-life of about 3-4 mo. which is comparable to values observed by Cunningham (1979) and Correll and Wu (1982) for similar microcosms. This degradation rate is considerably slower than the rate reported by Jones et al. (1982a) using smaller experimental systems exposed to ambient temperature and light. Concentrations of the herbicide taken up by either *P. perfoliatus* or *M. spicatum* were below our limits of detection (1.0 μg/kg) *in all experiments*, and concentrations were also low for the sediments. Thus, very little of the applied atrazine was sorbed or degraded, so that concentrations were relatively constant.

Atrazine Effects on Photosynthesis and Respiration

Measurements of P_a and R_n for these SAV microcosms were initiated 2 wk after planting of the communities, and they were continued for 5 wk prior to treatment. During this pretreatment phase, P_a and R_n (Fig. 5 and 6, Appendix Tables 1 A-D, 5 A-D) exhibited a general increase as the communities became established. Values for P_a were consistently higher in the *M. spicatum* communities, and this may be attributable to the 30% *greater quantum efficiency* of this plant compared to *P. perfoliatus* (Kemp et al. 1981). Since the analysis of experimental effects *compares treatment* level to control for all dates following initial dosing, some consideration was given to the comparability of these communities prior to treatment. Analysis of covariance indicated that the slopes of increasing P_a versus time for all aquaria in a given experiment were not significantly different (Table 2). The mean pretreatment values of P_a were, however, statistically different (Table 2), so that all post-treatment values could be normalized to controls according to the pretreatment ratio of mean P_a for a given microcosm to mean P_a of controls (referred to as "intercept adjustment"). We present ANOVA results for these data both with and without this adjustment.

The effects of atrazine on P_a of *P. perfoliatus* and *M. spicatum* are summarized in Table 3. At atrazine ≥ 50 ppb *there was significant depression* of P_a for *P. perfoliatus* in all post-treatment weeks (8-11), while at 5 ppb the *effects* were significant in weeks 9 and 10 but not 8 and 11. The vehicle controls exhibited a small but significant decrease in P_a for week 9, with an effect in week 8 only apparent without intercept adjustment. *Myriophyllum spicatum* P_a was less sensitive to the negative effects of *atrazine, and in fact there* was a significant enhancement of P_a over vehicle controls at 5 ppb. Similar growth stimulation at low concentration of photosynthetic inhibitor herbicides has been observed for various terrestrial weeds (Ries 1976; Hoffman and Lavy 1978) and submerged plants (Forney and Davis 1981; Correll and Wu 1982). The mechanism for this stimulation is unclear. However, two possible explanations are: first, utilization of nitrogen released during degradation of atrazine by these nutrient-limited plant populations (Kemp et al. 1980); and second, increased chlorophyll *a* production under atrazine stress (Cunningham 1979). A significant loss of P_a *was* evident at atrazine ≥ 50 ppb for the first 2 wk of treatment. However, in weeks 9 and 10 no *negative* effects

Table 1. Results of atrazine analysis of water, sediments and plant tissue at the end of the 1979 microcosm experiments#. Given are means ± standard deviation.

Sample	Water (μg/ℓ)	Sediment (μg/kg)	Plant Tissue (μg/kg)	Percent Recovery
50 ppb atrazine	42.3 ± 4.5	TR*	TR	84
100 ppb atrazine	89.6 ± 24.6	TR	TR	89
500 ppb atrazine	445 ± 57.6	1.4 ± 0.8	TR	89
1000 ppb atrazine	869 ± 128	2.4 ± 0.4	TR	87

#Control and 5 ppb samples were contaminnated prior to analysis.
*TR ≤ 0.1 μg/kg for sediments and 1.0 μg/kg for plant tissues.

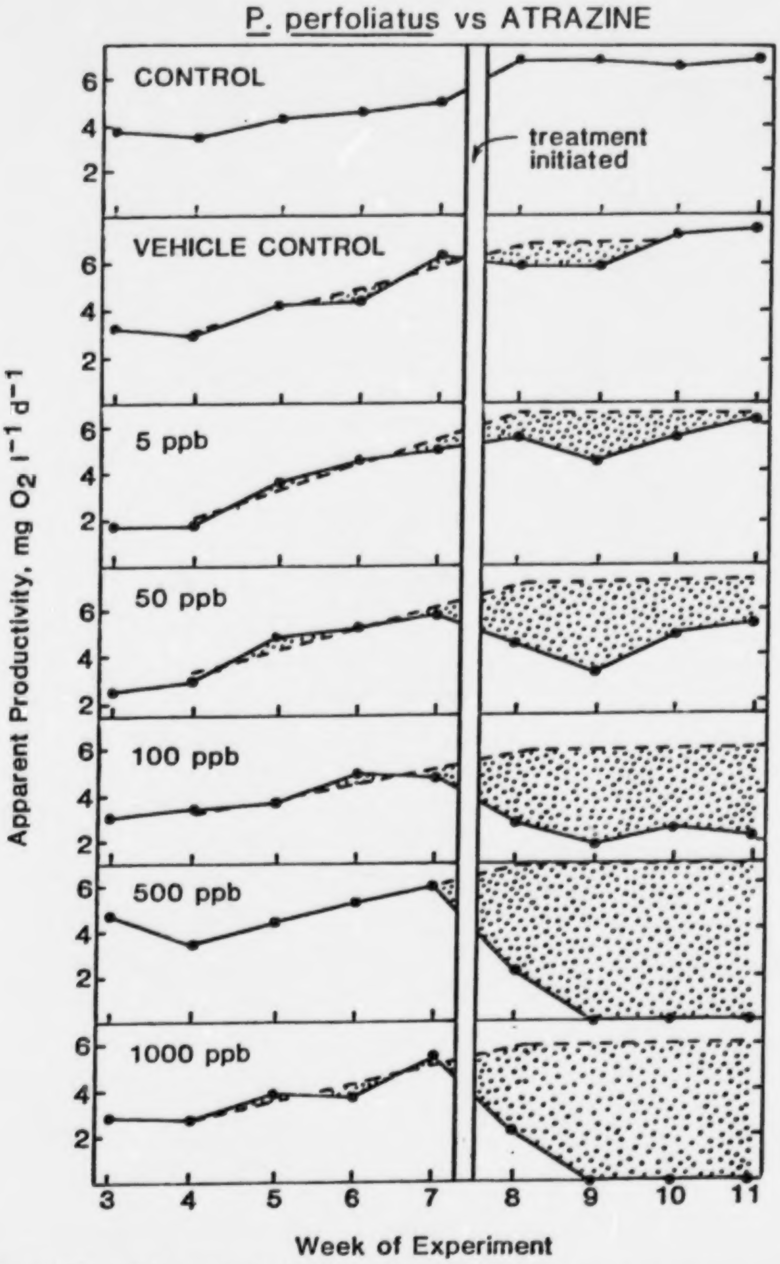

Figure 5. Mean of four weekly measurements of apparent O_2 production for microcosm communities containing Potamogeton perfoliatus exposed to 5 levels of atrazine dosage with two kinds of controls (untreated and treated with solvent only). Shaded areas represent approximate departures from control levels.

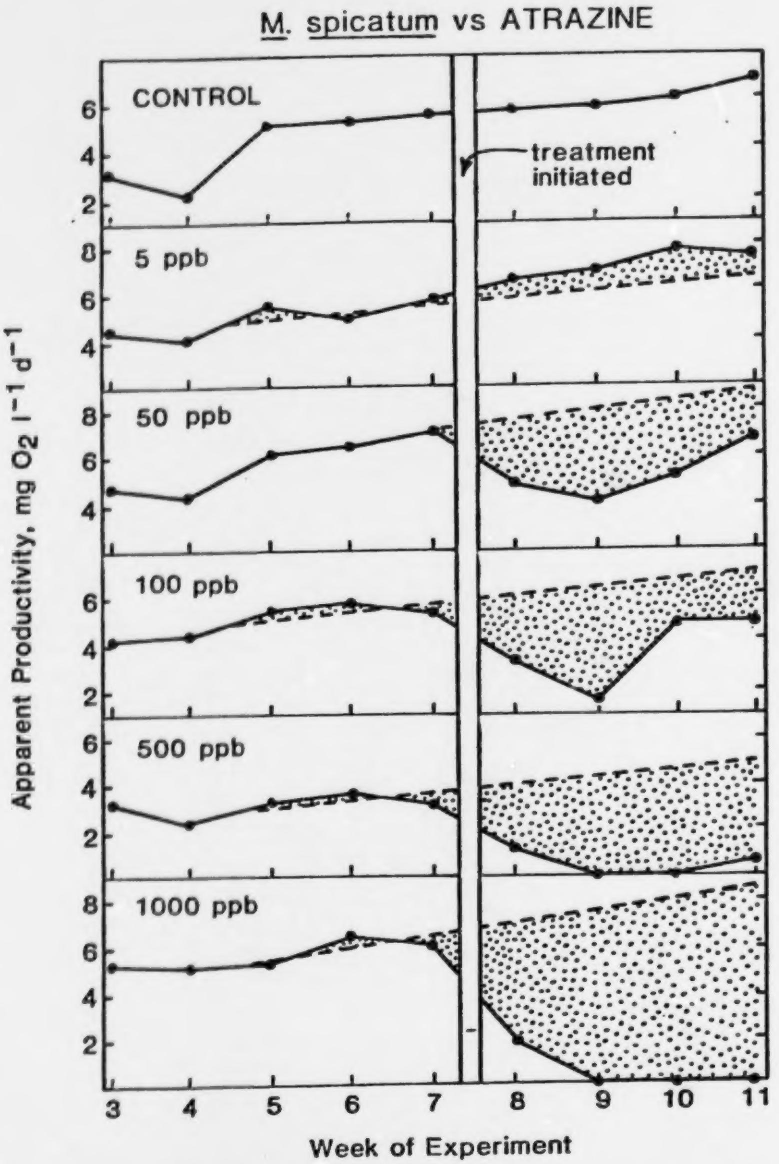

Figure 6. Mean of four weekly measurements of apparent O_2 production for microcosm communities containing Myriophyllum spicatum (see Fig. 5 caption) exposed to 5 levels of atrazine dosage with vehicle control (treated with solvents only).

Table 2. Summary of one-way analysis of variance and covariance for pretreatment behavior in experiments to test herbicide effects on *Potamogeton perfoliatus* and *Myriophyllum spicatum* apparent photosynthesis (P_a). Tests are for equality of slopes as P_a increased during pre-treatment and equality of mean pre-treatment values of P_a.†

Experiment	Equality of Slopes #	Equality of Cell Means*
Potamogeton vs. Atrazine	0.3645	0.0011*
Myrophyllum vs. Atrazine	0.8064	0.0000*
Potamogeton vs. Linuron	0.2270	0.0110*
Myrophyllum vs. Linuron	0.6561	0.0353*

†Table entries are probabilities that slopes or means are different.

#Values <0.05 indicate that there are significant differences ($p<0.05$) among pre-treatment slopes of P_a over time.

*Values <0.05 were taken to indicate that time-zero intercepts (i.e., initial P_a) were significantly different among individual microcosms.

were seen at concentration <100 ppb. Respiration generally followed P_a closely (Appendix Tables 1B and D, 5B and D) with some lag in response time, so that the ratio $P_a:R_n$ provided a less sensitive measure of stress.

The effects observed in this experiment at concentrations $\geq$50 ppb are similar to those reported previously for various *s*-triazine herbicides and submerged vascular plant species. For example, Sutton et al. (1969) found 40% loss of P_a for *E. canadensis* and *Myriophyllum sp.* after 4 d at 120 ppb atrazine, while Bielecki and Skrabka (1976) found similar effects for *Spirodela polyrhiza* after 24 h exposure to 100 ppb simazine, and Fowler (1977) reported 50% decrease in P_a of *Myriophyllum sp.* at 500 ppb of another triazine (DPX 2674) after 10 d. Correll and Wu (1982) observed a range of effects from 48% stimulation to 39% depression of P_a after 3-6 wk exposure to 75-650 ppb atrazine for 4 other SAV species (*P. pectinatus*, *Zanichellia palustris*, *Vallisneria americana*, *Zostera marina*). Recently, Walsh et al. (1982) has estimated the atrazine concentration for a 50% loss of P_a in the tropical seagrass, *Thalassia testudinum*, to be 320 ppb.

There is striking evidence of photosynthetic recovery for both *P. perfoliatus* and *M. spicatum* 3 wk after treatments of $\leq$50 ppb atrazine, and for *M. spicatum* even at 100 ppb (Figs. 5 and 6, Table 3). This recovery is similar to that reported by Cunningham et al. (1982) for *P. perfoliatus* at 100 ppb. What is so remarkable about this recovery is that it occurred in the face of relatively constant external aqueous concentrations of the herbicide. Jones et al. (1982b) have demonstrated the ability of *P. perfoliatus* exposed to 10-100 ppb atrazine to recover P_a with sequential washes in herbicide-free water, and this response was attributable to removal of the herbicide from the chloroplasts. In the present case, however, the mechanism of recovery must involve some sort of persistent detoxification, but its exact description awaits further investigation.

Phytoplankton production comprised a small to negligible portion of total community photosynthesis, representing <5% of community P_a for all but one occasion (when it was $\leq$15%). In fact, mean plankton P_a was negative for 25 of 42 occasions (Table 4). A possible explanation for this reduced phytoplankton production is nutrient limitation (Kemp et al. 1980). Concentrations of dissolved inorganic nitrogen remained low in all herbicide experiments (Table 5), with NH_4^+ generally below 3 μM and NO_3^- below 5 μM for all but the high herbicide treatments (>500 ppb). Nevertheless, there was a significant trend of reduced P_a and increased R_n in the plankton with increasing atrazine, particularly above 50 ppb. This pattern is consistent with various studies of *s*-triazine toxicity for algae (Walsh 1972; Pruss and Higgins 1974; Valentine and Bingham 1976; Hawxby et al. 1977). Since the walls of these experimental aquaria were maintained clean of algal periphyton, while sediments (Cunningham 1979) and plankton (Table 4) were generally heterotrophic, dawn-dusk DO increases in microcosm water probably represent slight underestimates of vascular plant P_a.

Atrazine Effects on Plant Biomass

Time-course patterns of *P. perfoliatus* biomass in response to atrazine

Table 3. Summary of one-way analysis of variance for post-treatment data in experiments to test atrazine effects on apparent photosynthesis (P_a) of 2 macrophyte species.*

Experiment	Treatment	Experimental Week Number 8	9	10	11
		No Intercept Adjustment			
Potamogeton vs. Atrazine	Control	0.650a	0.529a	0.556a	0.549a
	Vehicle Control	0.536b	0.301b	0.568a	0.610a
	5 ppb	0.505b	0.158c	0.416b	0.510a
	50 ppb	0.284c	-0.069d	0.307c	0.341b
	100 ppb	0.008d	-0.383e	0.066d	0.140c
	500 ppb	-0.286e	-0.341e	-0.348e	-0.198d
	1000 ppb	-0.196e	-0.517f	-0.291e	-0.160d
Myriophyllum vs. Atrazine	Vehicle Control	0.514a	0.353a	0.540a	0.588a,b
	5 ppb	0.617a	0.392a	0.674b	0.672a
	50 ppb	0.211b	-0.134b	0.394a	0.514b
	100 ppb	-0.024c	-0.253b	0.398a	0.323c
	500 ppb	-0.183d	-0.389b	-0.122c	-0.083d
	1000 ppb	-0.448e	-0.547b	-0.268d	-0.321e
		Intercepts Adjusted to Control			
Potamogeton vs. Atrazine	Control	0.602a	0.481a	0.508a,b	0.501a
	Vehicle Control	0.563a	0.328b	0.595a	0.637b
	5 ppb	0.505a	0.158c	0.416b	0.510a
	50 ppb	0.225b	-0.129d	0.248c	0.282c
	100 ppb	-0.124c	-0.473e,f	-0.066d	0.008d
	500 ppb	-0.334d	-0.565f	-0.396e	-0.246e
	1000 ppb	-0.209c	-0.396e	-0.304f	-0.173e
Myriophyllum vs. Atrazine	Vehicle Control	0.188a	0.027a	0.178a	0.262a
	5 ppb	0.399b	0.175a	0.457b	0.455b
	50 ppb	-0.060c	-0.405b	0.123a	0.243a
	100 ppb	-0.294d	-0.522b,c	0.128a	0.053c
	500 ppb	-0.270d	-0.476 b,c	-0.209c	0.170d
	1000 ppb	-0.734e	-0.833c	-0.554d	0.607e

*ANOVA tested against treatment for each week after treatment initiated. Values given are mean P_a ($g\ O_2 m^{-3} h^{-1}$) , and letters following each entry indicate significantly ($p<0.05$) different statistical group according to an SNK multiple range test (read vertically with treatment for each experiment).

Table 4. Summary of apparent plankton production (P_a) and dark respiration (R_n) in microcosm tanks containing macrophytic plants treated with atrazine. A negative sign (-) associated with P_a values denotes net O_2 consumption; positive R_n indicates O_2 consumption, while (-) indicates net O_2 evoluton. Given are means ± standard deviation with values in mg O_2 $\ell^{-1} d^{-1}$.

					Atrazine Treatments (ppb)				
Species	Date		Control	Vehicle Control	5	50	100	500	1000
P. perfoliatus	22/8/79	P_a	0.25 ± .71	0.27 ± .32	0.00 ± .27	0.52 ± .22	0.38 ± .11	0.21 ± .64	-0.22 ± .32
	(Week 5)	R_n	0.14 ± .40	0.09 ± .28	0.29 ± .34	0.32 ± .25	0.01 ± .14	+0.11 ± .50	0.17 ± .46
	30/8/79	P_a	-0.37 ± .44	-1.61 ± .73	-0.78 ± .14	-1.47 ± .26	-1.89 ± .32	-2.24 ± .56	-1.27 ± .58
	(Week 6)	R_n	0.48 ± .35	1.42 ± .38	0.73 ± .11	1.25 ± .10	1.62 ± .28	2.54 ± .28	0.91 ± .32
	12/9/79	P_a	0.38 ± .40	0.22 ± .08	-0.21 ± .08	0.20 ± .18	0.07 ± .15	-0.32 ± .11	-0.31 ± .20
	(Week 8)	R_n	0.06 ± .12	0.05 ± .12	0.15 ± .06	0.10 ± .20	0.17 ± .12	0.41 ± .07	0.34 ± .11
M. spicatum	22/8/79	P_a	0.25 ± .71	-0.06 ± .11	0.95 ± .37	0.33 ± .13	-0.16 ± .47	-0.29 ± .71	0.53 ± .88
	(Week 5)	R_n	0.14 ± .40	0.16 ± .13	0.36 ± .33	0.14 ± .33	0.36 ± .25	0.47 ± .32	0.38 ± .38
	30/8/79	P_a	-0.37 ± .44	-1.41 ± .89	-0.43 ± .37	-1.86 ± .75	-2.06 ± .19	-2.33 ± .30	-10.70 ± .32
	(Week 6)	R_n	0.48 ± .35	1.06 ± .48	0.80 ± .26	1.47 ± .49	1.56 ± .32	1.74 ± .17	6.05 ± 3.03
	12/9/79	P_a	0.38 ± .40	-0.15 ± .06	0.25 ± .22	0.03 ± .21	-0.13 ± .34	-0.14 ± .28	-0.10 ± .21
	(Week 8)	R_n	0.06 ± .12	0.17 ± .10	+0.05 ± .15	0.16 ± .31	0.28 ± .16	0.17 ± .17	0.33 ± .26

Table 5. Summary of ambient nutrient concentrations (ammonium, NH_4^+; nitrate, NO_3^-) in microcosms containing 2 species of macrophytes, treated with various concentrations of 2 herbicides. Given are means ± standard deviation in units of μM.

Species	Herbicide	Date	Treatment Control + Vehicle Control + 5.0 ppb		50 ppb + 100 ppb		500 ppb + 100 ppb	
			NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
P. perfoliatus	Linuron	18/6/80 (Week 6)	1.22 ± 0.57	1.72 ± 2.12	2.55 ± 1.29	0.31 ± 0.25	1.54 ± 0.78	5.33 ± 8.37
		30/7/80 (Week 12)	2.05 ± 0.96	0.46 ± 0.29	2.35 ± 1.31	0.38 ± 0.25	2.66 ± 1.91	21.90 ± 0.00
		4/9/80 (Week 17)	0.24 ± 0.33	0.27 ± 0.21	<0.10	0.30 ± 0.28	<0.10	0.17 ± 0.09
M. spciatum	Linuron	18/6/80 (Week 6)	2.80 ± 0.95	2.23 ± 2.42	2.57 ± 1.17	3.22 ± 3.29	3.54 ± 2.01	1.16 ± 1.05
		30/7/80 (Week 12)	1.44 ± 0.3	0.34 ± 0.24	2.01 ± 0.86	3.48 ± 6.32	4.65 ± 3.87	10.69 ± 9.73
		4/9/80 (Week 17)	0.23 ± 0.40	0.38 ± 0.16	-	-	0.00 ± 0.00	0.23 ± 0.00
P. perfoliatus	Atrazine	18/6/80 (Week 6)	2.10 ± 1.71	5.75 ± 4.52	1.81	1.88 ± 2.64	-	-
		30/7/80 (Week 12)	1.60 ± 0.74	0.26 ± 0.16	2.02 ± 0.61	0.36 ± 0.10	-	-
		4/9/80 (Week 17)	0.02 ± 0.07	0.18 ± 0.06	0.09 ± 0.16	0.17 ± 0.03	-	

treatment are presented in Fig. 7. For the control microcosms net production of above-ground biomass exhibited a logistic growth pattern, with an initial lag phase of 0.6 gdw $m^{-2}d^{-1}$, an exponential phase of 2.1 gdw $m^{-2}d^{-1}$, and an incipient plateau of 1.0 gdw $m^{-2}d^{-1}$. These correspond well (assuming O_2 = dry weight = 1.0) to apparent photosynthesis levels of 1.2, 1.5 and 1.8 $gO_2m^{-2}d^{-1}$, respectively. The pattern of *P. perfoliatus* growth was unaffected by 5 ppb atrazine, and while some effect is evident after 4 weeks of exposure at 50 ppb, it was not significant)ANOVA; SNK tests). Atrazine $\geq$100 ppb resulted in significant loss of biomass at week 11, although the effect at week 8 was significant only at concentrations $\geq$500 ppb. Forney and Davis (1981) also found significant loss of biomass for *P. perfoliatus* after 4 wk exposure to 100 ppb atrazine, while Fowler (1977) reported 20-80% loss of plant vigor (visually discerned) for *P. pectinatus* at 125 ppb of another *s*-triazine, (DPX 3674), for 30-54 d. The submersed aquatic plant, *V. americana* is apparently more sensitive to herbicide stress, where Correll and Wu (1982) observed significant increases in mortality after 47 d at 12 ppb atrazine.

Biomass production by *M. spicatum* (Fig. 8) exhibited more of an exponential trend in control systems, with highest rates during the final interval being about 1.7 gdw $m^{-2}d^{-1}$. The same growth sequence was exhibited in all treatments through 100 ppb, with no significant treatment effect. Only at 500 and 1000 ppb were biomass levels significantly reduced compared to controls. Thus, biomass data suggest (as did P_a measurements) that this exotic species of SAV was more tolerant of herbicide stress than was the native. However, unlike the P_a data, there was no evidence in biomass data of a growth stimulation at the low treatment (5 ppb).

The biomass estimates in Figs. 7 and 8 are based on direct measurements for the first and fourth dates (the first was based on wet weights of plants inserted into aquarium sediment, and the fourth was from final harvests), but indirect methods were used for the two intermediate observations. These are based on length-weight relations developed during the course of the experiment. In an earlier study (Cunningham et al. 1982), a significant decrease in slope of this relation was observed at atrazine treatments of 100-1000 ppb for *P. perfoliatus*. In the present study, the atrazine treated plants similarly exhibited a reduced slope (Fig. 9), although the difference was not significant, perhaps due to the shorter water columns in these microcosms compared to those used by Cunningham et al. (1982). These data are from experimental week 8, and the few plants taken from 500 and 1000 ppb atrazine treatments followed the same general trend as those from 50-100 ppb. A pronounced difference in slope occurred for *M. spicatum* treated with atrazine, and eventhough fewer samples were taken for this species, the slopes were significantly separated ($p<0.05$) (Fig. 10). The mechanism for this stem elongation under atrazine stress is unclear, although it is similar to a well-established shade response (Cunningham et al. 1982).

Linuron Experiments

Linuron Concentrations

Concentrations of linuron were dramatically reduced during the 4 wk experi-

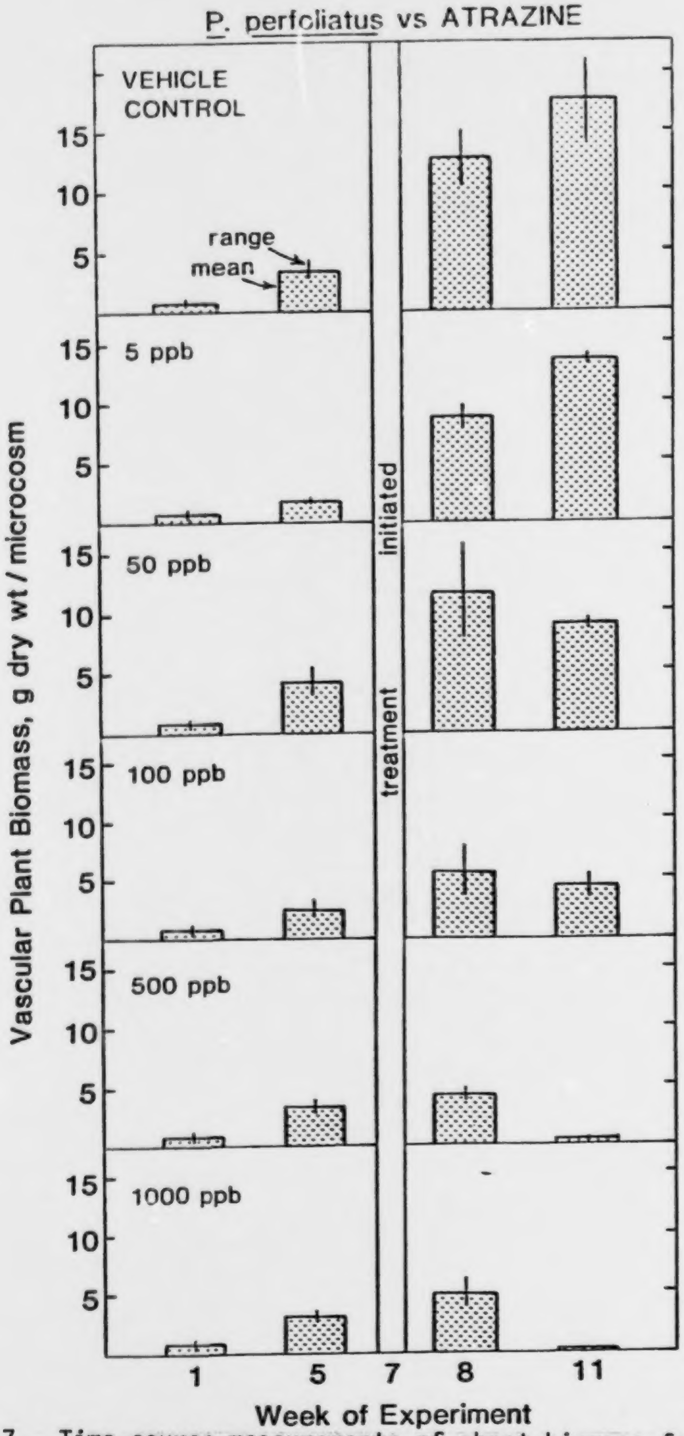

Figure 7. Time-course measurements of shoot biomass for Potamogeton perfoliatus exposed to 5 levels of atrazine dosage with vehicle control (solvent treatment). Initial biomass was estimated by measuring wet weight and using wet-dry weight regression; middle two values were estimated by measuring stem lengths and using a length-weight regression; final biomass estimated by harvest. Given are mean and range of two replicate microcosms.

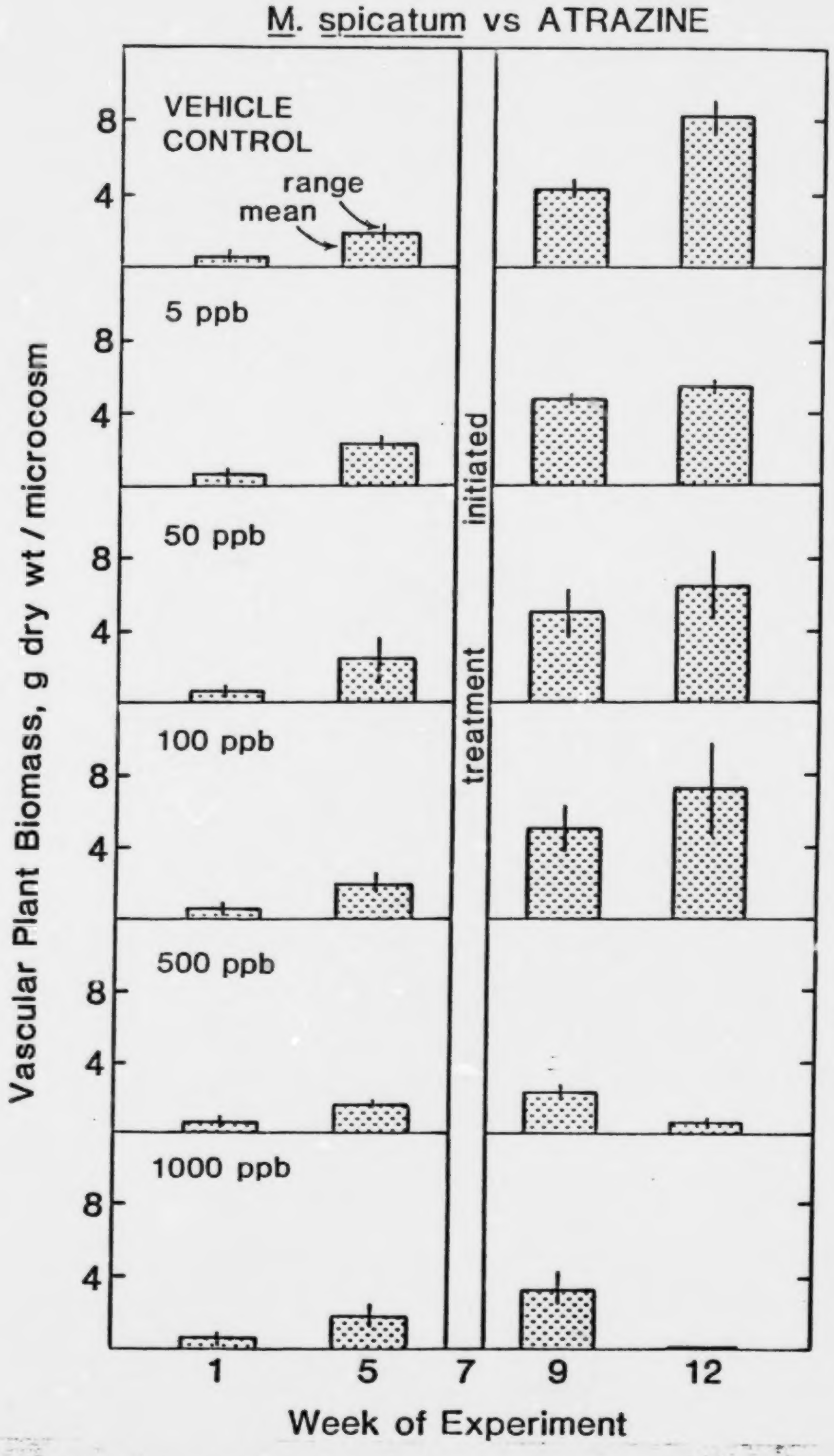

Figure 8. Time-course measurements of shoot biomass (see Fig. 7 caption) for Myriophyllum spicatum exposed to 5 levels of atrazine dosage with vehicle control (treated with solvent only).

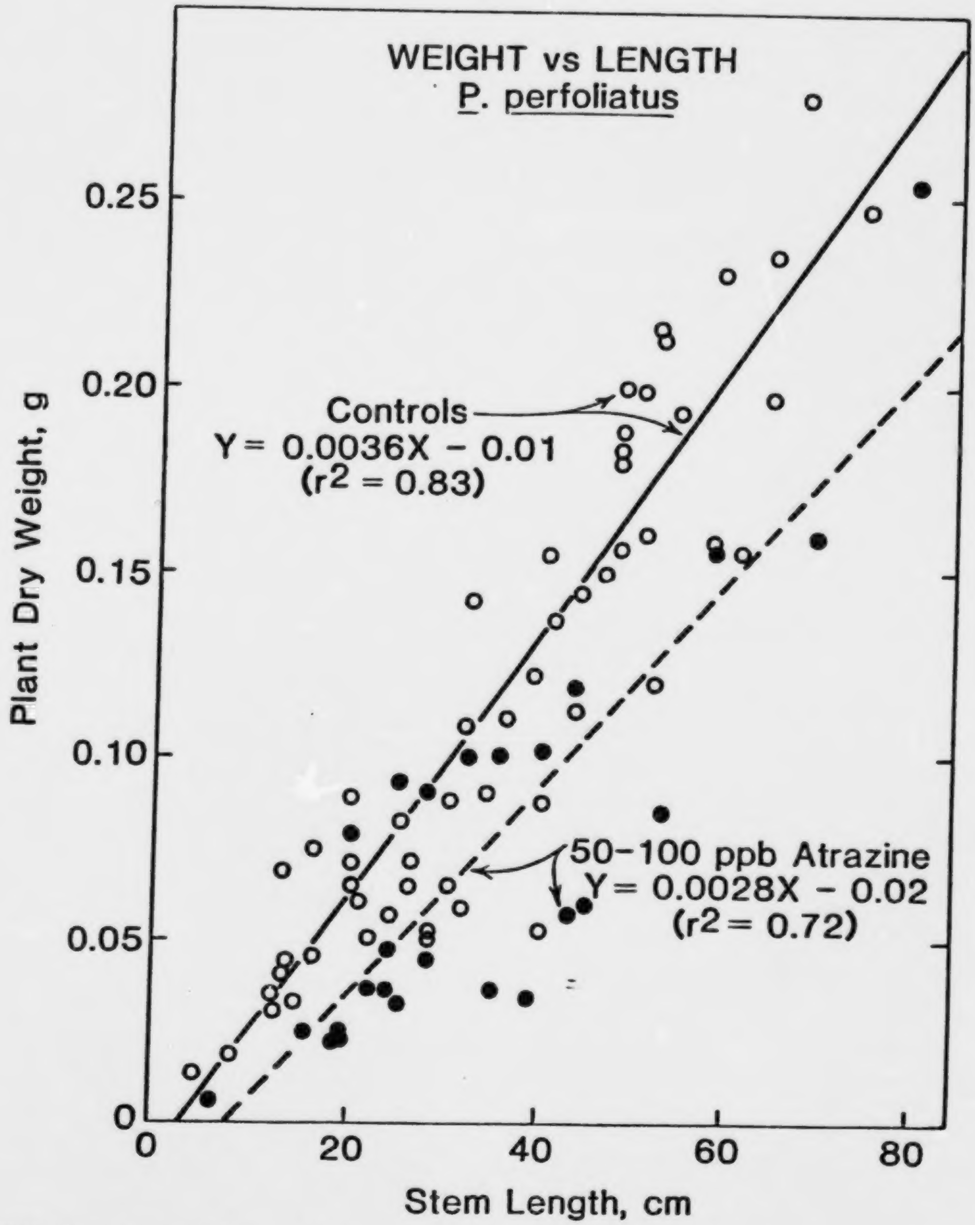

Figure 9. Regressions of stem length versus weight for Potamogeton perfoliatus in microcosms untreated and exposed to atrazine.

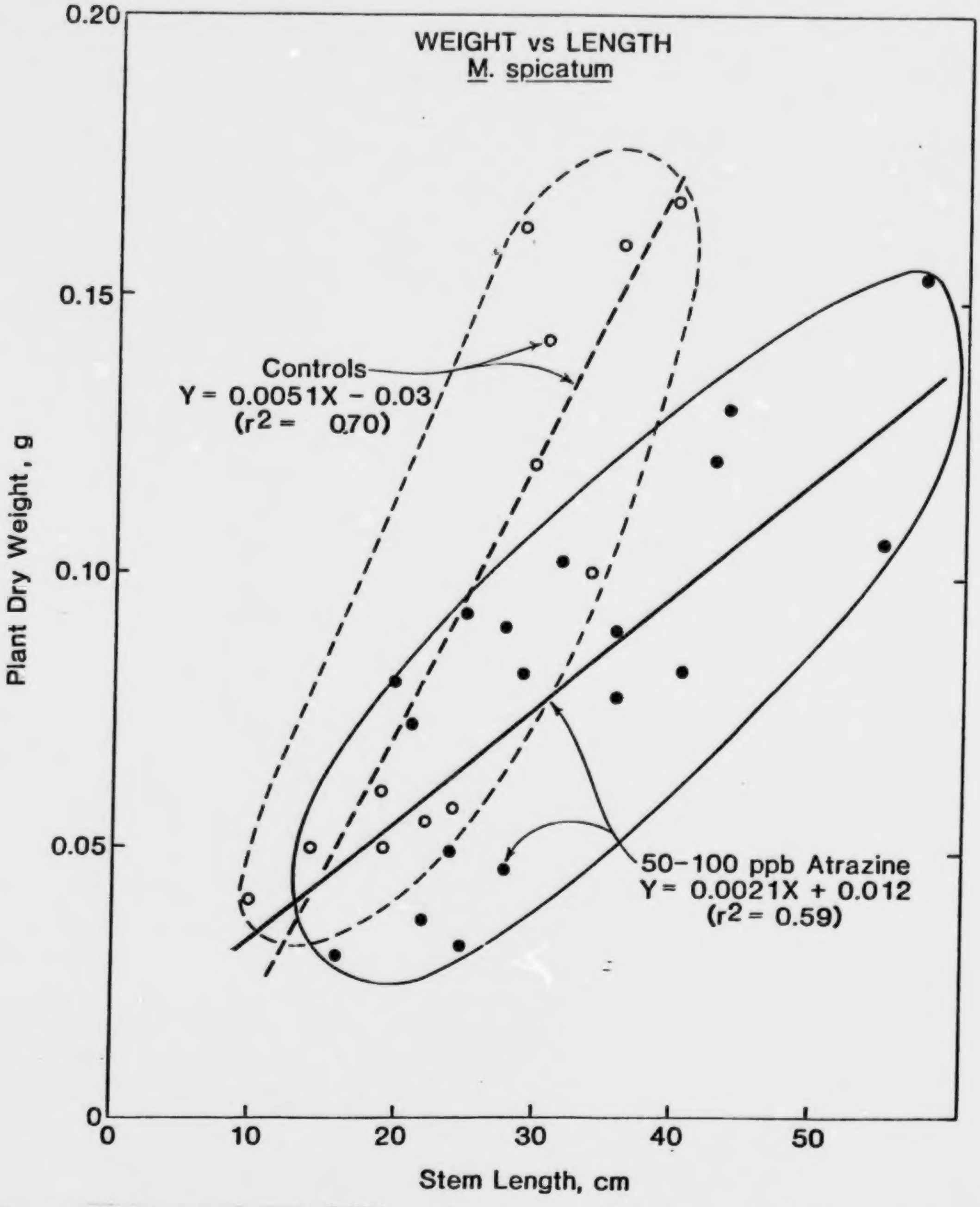

Figure 10. Regressions of stem length versus weight for Myriophyllum spicatum in microcosms untreated and exposed to atrazine.

mental period, where only about 15% of the original dose remained to the end (Table 6). This amounts to a half-life (time until 50% loss) of about 1-2 wk, for a first-order decay process. The degradation of linuron was about 10 times more rapid than that observed for atrazine in comparable experiments. While there have been no previous studies of linuron degradation in estuarine systems, the literature for agricultural soils confirms the relatively labile nature of this compound (Kemp et al. 1982). The amount of linuron sorbed to sediments in these aquaria was similar to that in the atrazine experiment, but partition coefficients (soil concentration:aqueous concentration) were about 10 times higher for linuron, which is consistent again with the agronomy literature (Kemp et al. 1982).

Linuron Effects on Photosynthesis and Respiration

After an initial drop from week 3 to 4, P_a and R_n for both P. perfoliatus and M. spicatum controls steadily increased until week 8, after which no change was apparent (Figs. 11 and 12). Values of P_a and R_n were similar for both species (Appendix Tables 4 A-D and 6 A-D). As with the atrazine experiment, we considered the comparability of pretreatment values of P_a by means of an analysis of covariance to compare the slope of increasing pretreatment P_a and the mean P_a at time of treatment. We determined that the pretreatment slopes of P_a versus time were not significantly different, but mean values of P_a were (Table 2). Therefore, we analyzed metabolic data both with and without adjusting post-treatment means to account for this departure from pretreatment controls (referred to as "intercept adjustment").

The results of sequential one-way ANOVA (with SNK) tests for differences in P_a among linuron treatments are summarized in Table 7. No effect on P. perfoliatus photosynthesis was evident at 5 ppb linuron, while concentrations $\geq$50 ppb always resulted in significant depression of P_a compared to controls, and even greater effect occured at linuron $\geq$500 ppb. Intercept adjustment did not alter statistical interpretation. For M. spicatum significant loss of P_a occurred at linuron $\geq$100 ppb and occasionally at 50 ppb as well. While there was a slight stimulation of P_a for M. spicatum at 5 ppb, this effect was not significant. Respiration generally followed a pattern similar to that for P_a, indicating a close coupling of anabolic and catabolic processes (Appendix Tables 4B and D, 6B and D). However, effects on respiration were less severe, and the ratio $P_a:R_n$ indicated that growth would be depressed in response to linuron treatment, although the effect would be somewhat buffered.

Photosynthesis for both species exhibited a significant recovery trend (Figs. 11 and 12) at all treatments $\leq$100 ppb (determined by significance of the slope of P_a versus time). This pattern is similar to that observed for atrazine treated plants. However, the mechanisms for recovery are probably quite different since linuron degradation occurred rapidly compared to atrazine in these microcosms. About 36% of the original dose had already been lost just 1 wk after treatment. Correspondingly, at the 50 ppb treatment about 30% recovery of P_a was apparent after 1 wk of exposure. Thus, while recovery of P_a in the atrazine experiments probably involved some sort of enzymatic detoxification, simple degradation of dissolved linuron in these experimental systems may account for the observed return of photosynthesis.

Table 6. Results of linuron analysis of water, sediments and plant tissues at the end of the 1980 microcosm experiments. Given are means ± standard deviation.

Sample	Water (μg/ℓ)	Sediment (μg/kg)	Plant Tissue (μg/kg)	Percent Recovery
Control	0	0	0	--
Vehicle control	0	0	0	--
5 ppb linuron	1.1 ± 0.2	TR*	TR	22
50 ppb linuron	4.4 ± 3.1	TR	TR	9
100 ppb linuron	12.8 ± 5.6	TR	TR	13
500 ppb linuron	69.7 ± 38.7	1.0 ± 0.5	TR	14
1000 ppb linuron	100.0 ± 27.9	1.4 ± 0.8	TR	11

*TR ≤ 0.1 μg/kg for sediments and 1.0 μg/kg for plant tissue.

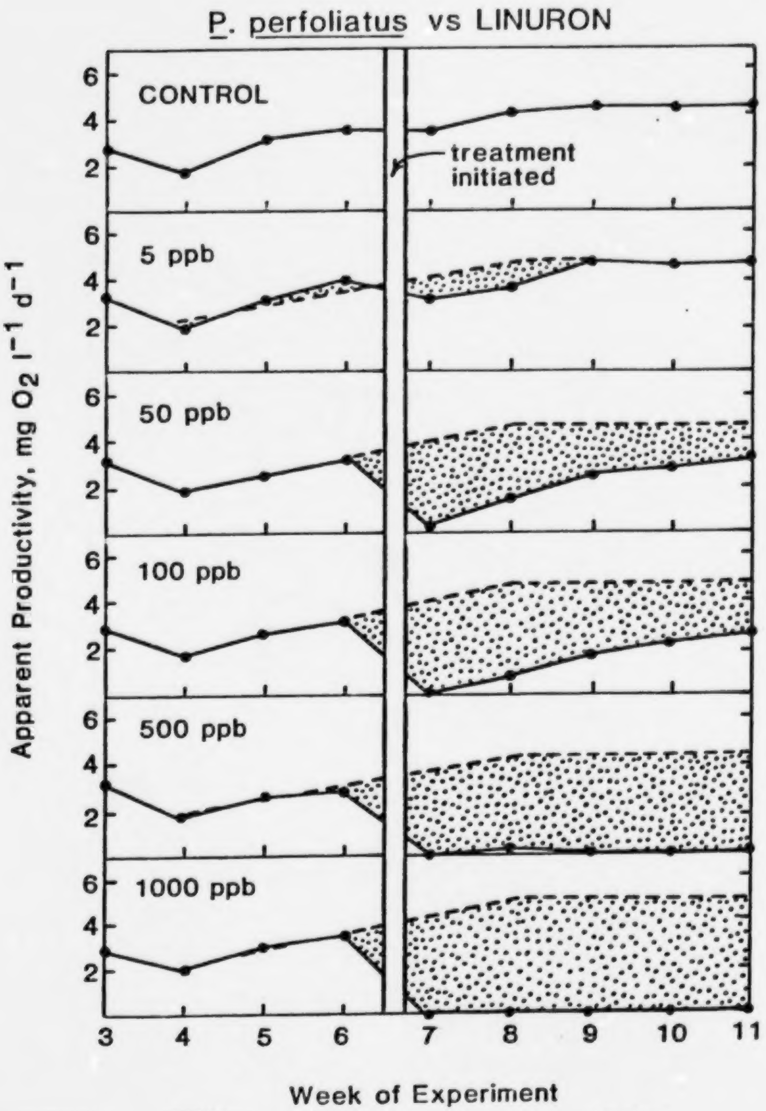

Figure 11. Mean of five weekly measurements of apparent O_2 production for microcosm communities containing Potamogeton perfoliatus exposed to 5 levels of linuron dosage with one control (see Fig. 5 caption).

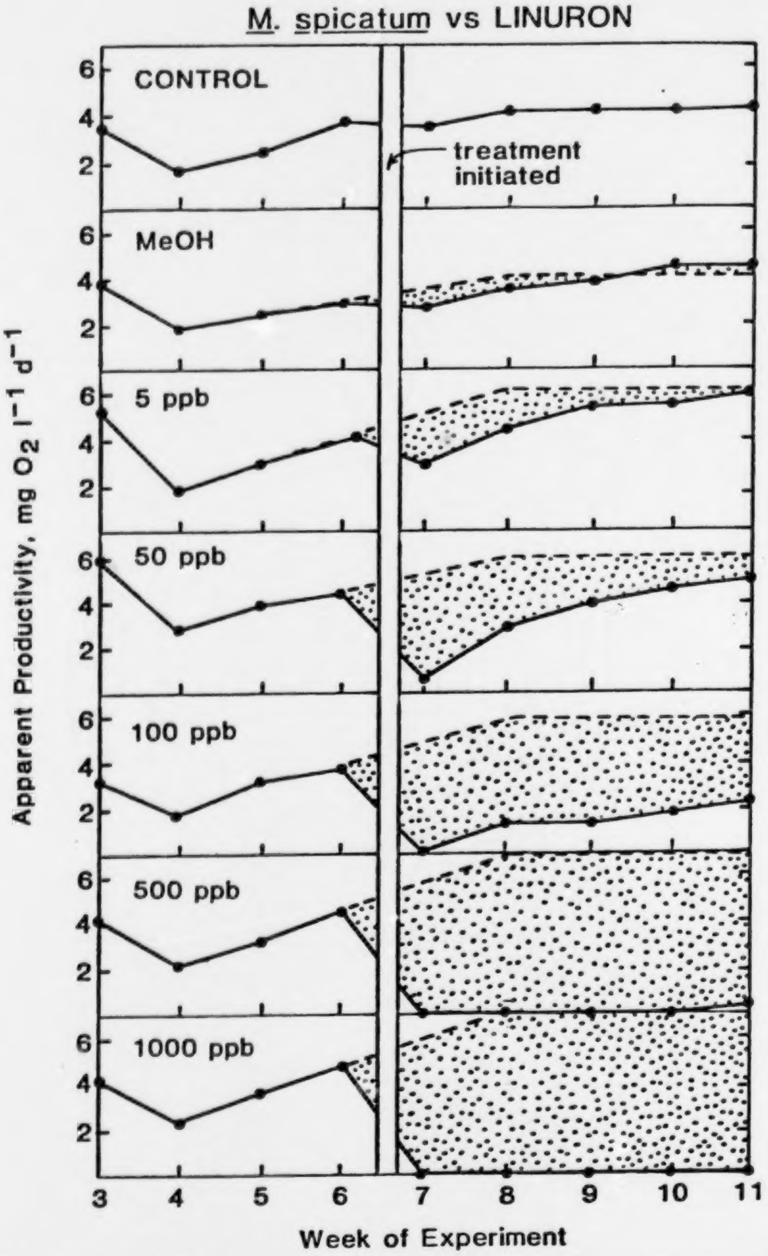

Figure 12. Mean of five weekly measurements of apparent O_2 production for microcosm communities containing Myriophyllum spicatum (see Fig. 5 caption) exposed to 5 levels of linuron dosage with two kinds of controls (untreated and treated with solvent only).

Table 7. Summary of one-way analysis of variance for post-treatment data from experiments to test linuron effects on apparent photosynthesis (P_a) of 2 submerged macrophyte species.

Experiment	Treatment	Experimental Week Number				
		7	8	9	10	11
No Intercept Adjustment						
Potamogeton vs. Linuron	Control	0.239a	0.342a	0.371a	0.371a	0.412a
	Vehicle Cont.	0.218a	0.310a	0.457a	0.488a	0.519a
	5 ppb	0.122a	0.132a	0.387a	0.431a	0.391a
	50 ppb	-0.111b	-0.090b	0.165b	0.192b	0.242b
	100 ppb	-0.243b	-0.202b	0.075b	0.106b	0.164b
	500 ppb	-0.264b	-0.253b	-0.147c	-0.135c	-0.111c
	1000 ppb	-0.279b	-0.313b	-0.246c	-0.216c	-0.203c
Myriophyllum vs. Linuron	Control	0.218a	0.339a,b	0.407a	0.519a	0.505a,b
	Vehicle Cont.	0.493b	0.331a,b	0.576b	0.587a	0.596a
	5 ppb	0.384b	0.425a	0.691b	0.763b	0.794c
	50 ppb	-0.081c	0.139b	0.365a	0.374c	0.422b
	100 ppb	-0.179c	0.088c	-0.050c	0.136d	0.160d
	500 ppb	-0.144c	-0.141c	0.016c	-0.005e	0.000e
	1000 ppb	-0.156c	-0.083c	-0.056c	-0.046e	-0.046e
Intercepts Adjusted to Control						
Potamogeton vs. Linuron	Control	0.282a	0.385a	0.414a	0.414a	0.455a
	Vehicle Cont.	0.394a	0.486a	0.628b	0.665b	0.695b
	5 ppb	0.272a	0.282a	0.536b	0.581b	0.5407a
	50 ppb	-0.035b	-0.014b	0.241c	0.267c	0.318c
	100 ppb	-0.213b	-0.173b	0.102c	0.135c	0.193d
	500 ppb	-0.170b	-0.159b	-0.052d	-0.042d	-0.0177e
	1000 ppb	-0.139b	-0.174b	-0.107d	-0.077d	-0.064e
Myriophyllum vs. Linuron	Control	0.179a	0.300a	0.368a	0.479a,b	0.467a,b
	Vehicle Cont.	0.283a	0.121a	0.367a	0.377a,b	0.386b
	5 ppb	0.143a	0.184a	0.450a	0.522a	0.553a
	50 ppb	-0.112b	0.108a	0.335a	0.343b	0.391b
	100 ppb	-0.232b,c	-0.141b	-0.003b	0.083c	0.107c
	500 ppb	-0.339c	-0.336b	-0.211c	-0.200d	-0.195d
	1000 ppb	-0.354c	-0.282b	-0.255c	-0.244d	-0.246d

*ANOVA tested against treatment for each week after treatment initiated. Values given are mean P_a (g O_2 $m^{-3}h^{-1}$) and letters following each entry indicate significantly different ($p<0.05$) statistical groups according to SNK multiple range test (read vertically with treatment for each experiment).

Overall, the effects of linuron treatment on productivity of these two plant species are remarkably similar to those observed for atrazine exposure. Few previous studies have examined linuron toxicity for aquatic plants. However, Bielecki and Skrabka (1976) have shown that short-term (24 h) losses of photosynthesis by *Spirodela polyrrhiza* were similar for linuron and simazine (an *s*-triazine closely related to atrazine), although slightly less severe for the latter compound. While the modes of action for these two herbicides are somewhat different, as with atrazine both are photosynthetic inhibitors. The similarity of effects between linuron and the *s*-triazines is consistent with a recent study by O'Brien and Prendeville (1979) which demonstrated that linuron and simazine induced identical rates of electrolyte leakage from the cell membrane of *Lemna minor*. Toxicities of linuron and atrazine for a wide range of terrestial weeds have also shown to be relatively comparable (Stevenson and Confer 1978).

Linuron Effects on Plant Biomass

At the end of this experiment total plant biomass was harvested from each microcosm, and these data are summarized in Fig. 13. For *P. perfoliatus* there was a clear trend of decreasing biomass with treatment at herbicide concentrations $\geq$50 ppb. No effect was evident at 5 ppb, but significant loss of plant material occurred at $\geq$50 ppb ($p \leq 0.05$, 1-way ANOVA, SNK). In fact, treatment effects separated into 3 groups (control and 5 ppb, 50 and 100 ppb, and 500 and 1000 ppb). There was a small but insignificant increase in biomass for *M. spicatum* at 5 ppb, although no such effect was apparrent in P_a data. A trend similar to that for *P. perfoliatus* occurred for *M. spicatum*, with mean biomass decreasing as treatments increased from 50-1000 ppb, but significant losses ($p < 0.05$) were seen only at linuron $\geq$500 ppb. At 100 ppb the effect was marginally significant ($p \leq 0.20$). It appears that plant biomass is a less sensitive index of linuron stress than P_a, possible because P_a and R_n tend to become adjusted, minimizing effects on plant growth.

IMPLICATIONS: HERBICIDES AND ESTUARINE VASCULAR PLANTS

Relative Phytotoxicity of Herbicides for Plants

The relationships between herbicide dosage and plant production or biomass can be expressed in terms of an exponential dose-response model (Bliss 1970; Horowitz 1976). In Fig. 14 we present mean P_a as a percent of vehicle control plotted versus the logarithm of nominal herbicide concentration for the four experiments performed in this study. This model provides a good description for the results of two experiments (atrazine/*M. spicatum* and linuron/*P. perfoliatus*), with $r^2 \geq 0.99$. While some version of the logistic model might have been more appropriate for the other two experiments (Bliss 1970; Streibig 1980), the exponential model, nonetheless, accounted for > 94% of the variance in those data (Fig. 14).

Overall, the dose-response relations for all four experiments were similar with slopes and intercepts ranging from 36.8-48.9 and 110.5-147.4, respectively. However, several differences are worth noting. The generally greater sensitivity of *P. perfoliatus* to both herbicides is evident here by the

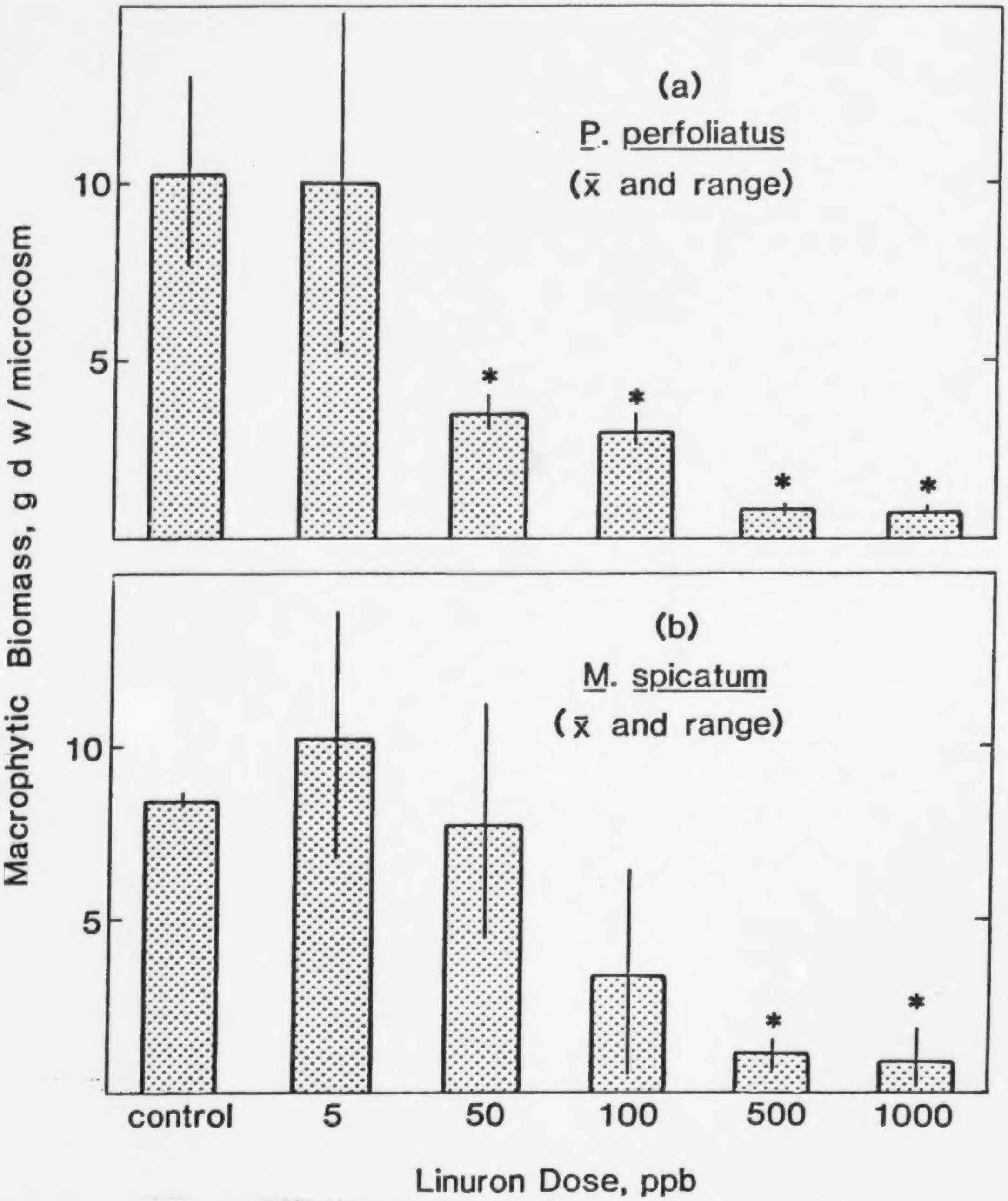

Figure 13. Shoot biomass for a) P. perfoliatus and b) M. spicatum harvested after 5 weeks exposure to 5 levels of linuron dosage with untreated controls. Given are means and ranges for duplicate microcosms. Asterisks indicate significant ($p<0.05$) differences between treatments and controls.

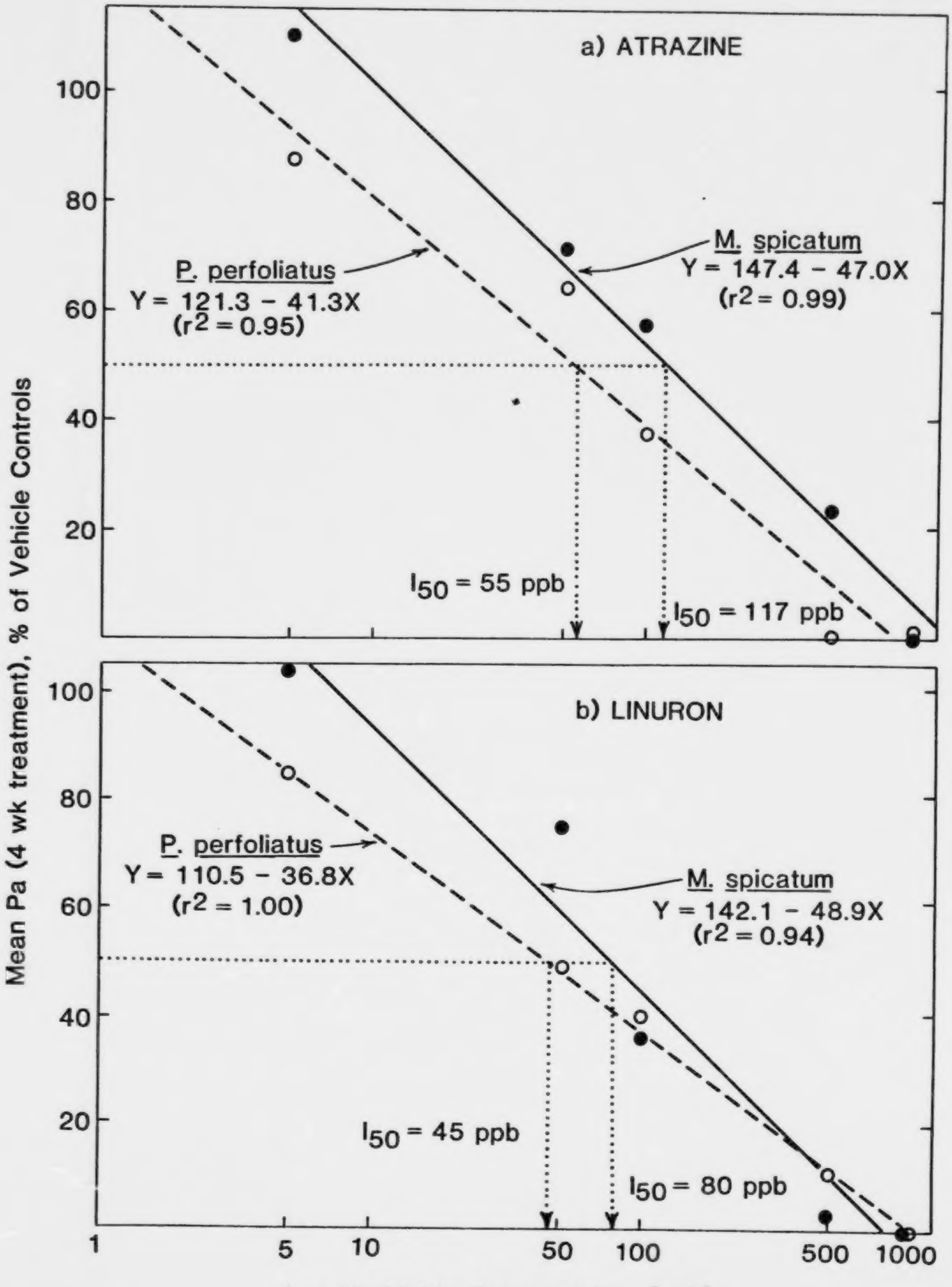

Fig. 14. Regressions of mean apparent O_2 production (as a % of control) over the first 4 weeks of treatment versus log of herbicide concentration for P. perfoliatus and M. spicatum treated with a) atrazine and b) linuron. Also shown are values of I_{50} (herbicide concentrations predicted to cause 50% photosynthetic inhibition) for each species and herbicide.

fact that mean P_a's for this species were lower than for *M. spicatum* at all but the highest herbicide concentrations. The apparent greater tolerancce of *M. spicatum* to atrazine and linuron exposure and the actual stimulation of its production at low concentrations might lead to some speculations concerning the proliferation of this species in upper Chesapeake Bay prior to the general die-back of all submerged plants (Bayley et al. 1978). Using I_{50} (herbicide concentration producing 50% inhibition) as an index of relative toxicity, we also find that linuron was slightly more effective than atrazine at reducing P_a for both plant species. Combining the results of all four experiments in this statistical model, we find a reasonably strong regression ($r^2 = 0.93$), with $I_{50} = 72$ ppb. The herbicide concentration inducing incipient (1%) inhibition ($I_{1.0}$) can be inferred from this model, and values of $I_{1.0}$ range from 2-11 ppb. Relationships between plant biomass (after a 5 wk treatment) and herbicide dosing concentration were also statistically significant for all experiments, with $r^2 \geq 0.93$ for all but the atrazine/*M. spicatum* study. Values for $I_{1.0}$ and I_{50} were similar for P_a and biomass (Table 8), which is not surprising given the physiological relation between production and final standing crop.

A few previous studies have used the same exponential dose-response model for herbicides and submerged vascular plants. Forney and Davis (1981) estimated the ranges of $I_{1.0}$ to be 1-14 ppb and that I_{50} to be from 53-907 ppb for *E. canadensis*, *V. americana* and *P. perfoliatus* exposed to atrazine using a variety of growth parameters to measure inhibition. They also found that *M. spicatum* was significantly more tolerant of herbicide stress than the other species. Walsh et al. (1982) reported that P_a for *T. testudinum* exhibited somewhat less sensitivity to atrazine exposure than observed in the present study for *P. perfoliatus* and *M. spicatum*, with I_{50} for the seagrass being about 320 ppb. The effects of atrazine on submerged plants reported by Correll et al. (1978) and Correll and Wu (1982) were not treated in a dose-response model. However, using a linear interpolation between % inhibition of P_a observed at 2 test concentrations, we estimate I_{50} values to have ranged from about 150-450 ppb, with the highest value for the temperate seagrass, *Z. marina*. Yet, at 12 ppb atrazine mortality of *V. americana* was estimated to be about 50% (i.e., $I_{50} = 12$ ppb) in a later experiment (Correll and Wu 1982).

In general the effects of herbicide treatment were more severe on P_a than on biomass (B); however, the pattern was similar in both cases. The ratio P_a/B provides a measurement of normalized plant growth or turnovr rate. In our study this ratio decreased with time in control microcosms, perhaps indicating a decrease in new growth. With herbicide treatment the ratio P_a/B exhibited a similar (but less pronounced) trend to that for either P_a or B separately.

Photosynthetic Recovery

In the above discussion we have used an operational definition of herbicide effect as the mean loss of P_a compared to vehicle controls over a 5 wk treatment period. However, we noted earlier that recovery of P_a was evident for all 4 herbicide/plant combinations at concentrations ≤ 100 ppb. In the case of atrazine this recovery occurred even in the face of relatively constant aqueous concentrations external to the plant tissue. Thus, integrated over an entire

Table 8. Summary of dose-response relations for herbicide treatment of submerged vascular plants.

Herbicide	Plant	Apparent Photosynthesis (P_a)*#			Final Biomass (B)*		
		r^2	$I_{1.0}$	I_{50}	r^2	$I_{1.0}$	I_{50}
Atrazine	*P. perfoliatus*	0.95	4	55	0.96	1	30
	M. spicatum	0.99	11	117	0.53	3	91
Linuron	*P. perfoliatus*	1.00	2	45	0.95	1	25
	M. spicatum	0.94	8	80	0.93	16	137
Combined	Combined	0.93	5	72	0.72	3	61

*Based on linear regression of P_a and B(express as % of vehicle controls) versus log herbicide concentration, with r^2 for least-square fit line and $I_{1.0}$ and I_{50} being herbicide concentration (ppb) associated with 1% and 50% inhibition, respectively.

#Mean values of P_a taken for the first 4 post-treatment weeks.

4 mo growing season, the effects might tend to be mitigated by regrowth. On the other hand, experiments using short-term (ca. 2 h) exposures of submerged plants to atrazine followed by sequential washes with herbicide-free water, revealed that complete photosynthetic recovery was not apparent for 1-2 d (Jones et al. 1982b). Whereas the former mode of recovery appears to involve some sort of enzymatic detoxification within the plant, the latter results simply from release of previously cell-bound atrazine. It seems that brief exposures to atrazine of about 1 h may result in some photosynthetic inhibition for 1-2 d, while loss of P_a from extended (1-5 wk) bathing in herbicide concentrations may be molified within a week. One consequence of this "buffering" effect is that both short and long-term dose-response experiments yield similar results in terms of I_{50} and other measures of inhibition (c.f. Jones et al. 1982b).

Field Concentrations of Herbicides and Their Effects

At the outset of this study the information on herbicide concentrations in Chesapeake Bay was sparse, although the few existing data suggested that concentrations in shallow coves could approach 10 ppb (Correll et al. 1978). Recently, Stevenson et al. (1983) have reported the results of an extensive survey of atrazine and linuron concentrations in various regions of Chesapeake Bay during the spring/summer seasons of 1980 and 1981. In this study herbicide concentrations (atrazine and linuron) were measured in water, suspended material and sediments at 23 littoral zone stations in the main bay area; at 8-10 stations along the salinity gradient of a major tributary (Choptank River); and at 4-5 stations along a runoff creek draining agricultural fields adjacent to the Choptank. Bay-wide samples were taken bi-monthly from April-September, Choptank samples were taken monthly during the same period and drainage from agricultural fields was monitored prior to, during and for 12-24 h following runoff events. Precipitation patterns in 1980 were similar to the ten-year (1970-80) average, while 1981 was characterized by intense rainfall in early May, with light sporadic precipitation in June and July (Kemp et al. 1982).

The ranges of atrazine and linuron concentrations observed in this survey are summarized in Table 9. A transect down the main axis of the estuary revealed that concentrations of either compound rarely exceeded 1 ppb, with highest values in the brackish reaches. A similar transect along the main salinity gradient of a major tributary (Choptank River/estuary), indicated concentrations in 1980 similar to those in the open bay, while in 1981 maximum values were 9.3 and 3.0 ppb, respectively, for atrazine and linuron. The high atrazine concentration was actually from a freshwater station just above the head of tide. In a creek and small estuarine cove connected to the Choptank and receiving direct agricultural runoff, peak concentrations approached 50 ppb atrazine and 20 ppb linuron, with highest values occurring during the May 1981 runoff events. Generally, concentrations in May were on the order of 1-5 ppb, and those > 10 ppb existed for only 2-8 h, being rapidly dissipated by dilution, adsorption and degradation. These concentrations are similar to those reported elsewhere for these coastal plain estuaries (Newby et al. 1978; Correll et al. 1978; Wu 1980; Zahnow and Riggleman 1980; Hershner et al. 1982).

Using these herbicide data, a relatively extreme runoff scenario can be

Table 9. Summary of ranges of atrazine and linuron concentrations observed in water from Chesapeake Bay and its primary and secondary tributaries.*

SAMPLING LOCATION	SAMPLING PERIOD	CONCENTRATION RANGE (ppb)	
		ATRAZINE	LINURON
1. Horn Point (Runoff Creek and Cove)	May-Jul 1980	0.1-18.3	0.0- 2.6
	May 1981	0.7-46.0	0.4-18.3
2. Choptank Estuary			
•Monthly Survey	May-Jul 1980	0.0- 0.8	0.1- 1.2
•Runoff Event	May 1981	0.2- 9.3	0.3- 3.0
3. Chesapeake Bay	Apr-Sep 1980	0.0- 1.2	0.0- 1.3

*Data summarized from Stevenson et al. (1983).

developed for submerged vascular plants growing in small coves and the overall effects of resulting herbicide concentrations on plant production can then be estimated using Fig. 14 and Table 8. In a wet spring such as that of 1981, we might find 2 major runoff events occurring in May spaced 2 wk apart and generating peak atrazine concentrations of 50 and 20 ppb, respectively, which last about 6 h, followed by baseline concentrations of 1-5 ppb until July. A subsequent runoff event in July (after the planting of a second grain crop) might result in peak linuron concentrations of 10 ppb for 6 h followed by 1-3 ppb for the remainder of the month. Such environmental conditions could be expected to result in the following sequence of P_a inhibition: May -- 50% loss of P_a for 2 d, 5% loss for 12 d, 30% loss for 2 d, 5% loss for 15 d; June -- 1% loss for 30 d; July -- 25% loss for 3 d, 5% loss for 28 d. Integrated over the entire 3 mo period, this would amount to a mean of 6% inhibition of P_a using a simplistic additive approach.

It is of interest to note that two recent papers investigating atrazine effects on submerged plant growth (Forney and Davis 1981; Correll and Wu 1982) reached what appear to be opposite conclusions concerning the possible threat posed by this herbicide for submerged plant populations in Chesapeake Bay. Whereas Forney and Davis (1981) stated that it is unlikely that atrazine "caused the die-off of plants in Chesapeake Bay," Correll and Wu (1982) suggested that "there is every reason to be concerned that herbicides such as atrazine may be seriously impacting submersed vascular plant populations in estuaries." Despite the apparent difference in these two conclusions the experimental results from these two studies are, as we discussed previously, similar. However, these two statements may not be as contradictory as they seem.

The data and analyses which we have provided here indicate that 1-10% loss of submerged plant production might occur in certain estuarine regions as a result of runoff of herbicides such as atrazine and linuron over the course of a growing season. Since these plant populations are stressed generally by reduced light and other perturbations (Kemp et al. 1982), such an herbicide-induced loss of production could add to the adversity of their environment, and therefore, the position of Correll and Wu (1982) would not be unreasonable. When these plants are existing at the margins of their adaptability range, small incremental stresses can undermine reproductive viability by shifting the physiological energy balance from net positive to negative. However, as indicated by Forney and Davis (1981), it is difficult to conclude from the results of this and other studies that herbicides, per se, caused the decline in Chesapeake Bay's submerged plants. Further studies examining the short and long-term effects of herbicide stress on SAV reproduction may be warrented to resolve this issue.

REFERENCES

1. Adams, S.M. 1976. Feeding ecology of eelgrass fish communities. Trans. Amer. Fish. Soc. 105:514-519.

2. Barsdate, R.J., M. Nebert, and C.P. McRoy. 1974. Lagoon contributions to sediments and water of the Bering Sea, pp. 553-575. In D.W. Hood and E.J. Kelly (eds.). Oceanography of the Bering Sea. Univ. Alaska Press, Fairbanks.

3. Bayley, S., V.D. Stotts, P.F. Springer, J. Steenis. 1978. Changes in submerged aquatic macrophyte populations at the head of the Chesapeake Bay, 1958-1975. Estuaries. 1:74-85.

4. Bielecki, K. and H. Skrabka. 1976. Effect of some herbicides on photosynthesis of Spirodela polyrhiza (Lemnaceae). Acta Agrobot. 29:59-68.

5. Bliss, C.I. 1970. Statistics in biology, Vol. 2. McGraw-Hill, London.

6. Cartwright, P.M. 1976. General growth response of plant, pp. 55-82. In L.J. Audus (ed.). Herbicides, physiology, biochemistry, ecology, Vol. 1. Academic Press, London.

7. Correll, D.L., J.W. Pierce and T-L Wu. 1978. Herbicides and submerged plants in Chesapeake Bay, pp. 858-877. In Amer. Soc. Civil Eng. (ed.). Coastal Zone-78. ASCE Publ.

8. Correll, D.L. and T.L. Wu. 1982. Atrazine toxicity to submersed vascular plants in simulated estuarine microcosms. Aquatic Bot. (In press).

9. Cunningham, J.J. 1979. Responses of microcosm communities containing submerged aquatic vegetation to herbicide stress. MS Thesis. Univ. MD, College Park. 66 pp.

10. Cunningham, J.J., W.M. Kemp, M.R. Lewis and J.C. Stevenson. 1982. Patterns of ecological and physiological response and recovery of the submerged macrophyte, Potamogeton perfoliatus, exposed to atrazine stress in estuarine microcosms. (Unpubl. MS.).

11. Forney, D.R. and D.E. Davis. 1981. Effects of low concentrations of herbicides on submersed aquatic plants. Weed Sci. 29:677-85.

12. Fowler, M.C. 1977. Laboratory trials of a new triazine herbicide (DPX 3674) on various aquatic species of macrophytes and algae. Weed Res. 17:191-195.

13. Hawxby, K., B. Tubea, J. Ownby and E. Basler. 1977. Effects of various classes of herbicides on four species of algae. Pestic Biochem. Physiol. 7:203-209.

14. Hershner C., K. Ward, J. Illowsky. 1982. The effects of atrazine on Zostera marina in the Chesapeake Bay, Virginia. Virginia Instit. Mar. Sci., Gloucester Pt., VA. US Environ. Protect. Agency, Annapolis, MD. No. R805953.

15. Hoffman, D.W. and T.L. Lavy. 1978. Plant competition for atrazine. Weed Sci. 26:94-99.

16. Horowitz, M. 1976. Application of bioassay techniques to herbicide investigations. Weed Res. 16:209-215.

17. Howard-Williams, C. 1981. Studies on the ability of a Potamogeton pectinatus community to remove dissolved nitrogen and phosphorus compounds from lake water. J. Appl. Ecol. 18:619-637.

18. Jones, T.W., W.M. Kemp, J.C. Stevenson and J.C. Means. 1982a. Degradation of atrazinne in estuarine water/sediment systems and selected soils. J. Environ. Qual. (In press).

19. Jones, T.W., W.M. Kemp, P.S. Estes and J.C. Stevenson. 1982b. Atrazine uptake, phytotoxicity, release and short-term recovery for the submerged aquatic plant Potamogeton perfoliatus. (Unpublished MS).

20. Kemp, W.M., M.L. Lewis, J.J. Cunningham, J.C. Stevenson, W.R. Boynton. 1980. Microcosms, macrophytes, and hierarchies: Environmental research in Chesapeake Bay, pp. 911-936. In J. Giesy (ed.). Microcosm research in ecology. ERDA Conf. 781101.

21. Kemp, W.M., J.J. Cunningham, J. Perron, S. Garabrandt, M.R. Lewis, R. Hanson, R. Twilley, W.R. Boynton, and J.C. Stevenson. 1981. Experimental observations of turbidity/light relations and their influence on submerged macrophytes in northern Chessapeake Bay, pp. 2-29 In W.M. Kemp et al. (eds.). Submerged aquatic vegetation in Chesapeake Bay. Univ. Md. Cntr. Env. Est. Res. Ref. No. HPEL 81-68, Cambridge, MD.

22. Kemp, W.M., J.C. Means, T.W. Jones and J.C. Stevenson. 1982. Herbicides in Chesapeake Bay and their effects on submerged aquatic vegetation. Univ. Maryland Center Enviorn. Est. Stud. Ref. No. 1295-HPEL. Horn Pt. Env. Labs, Cambridge, MD. 82 pp.

23. McConnell, W.J. 1962. Productivity relationships in carboy microcosms. Limnol. Oceanogr. 7:335-343.

24. Newby, L.C., R.A. Kahrs, K. Adams, and M. Szolics. 1978. Atrazine residues in the Chesapeake Bay. Proc. N.E. Weed Sci. Soc. 32:339 (Abstract).

25. O'Brien, M.C. and G.N. Prendeville. 1979. Effects of herbicides on cell membrane permeability in Lemna minor. Weed Res. 19:331-334.

26. Odum, H.T. and C.M. Hoskin. 1958. Comparative studies on the metabolism of marine waters. Publ. Inst. Mar. Sci. (Texas) 8:23-55.

27. Orth, R.J. 1977. The importance of sediment stability in seagrass communities, p. 281-300 In B.C. Coull (ed.) Ecology of marine benthos. Univ. S. Carolina Press, Columbia.

28. Orth, R.J. and K.A. Moore. 1981. Submerged aquatic vegetation of the Chesapeake Bay: Past, present and future. Trans. N. Amer. Widl. Nat. Res. 46:271-283.

29. Pruss, S.W. and E.R. Higgins. 1974. Effects of low levels of simazine on plankton algae in a non-stratified lake. Proc. N.E. Weed Contr. Counc. 28:124-131.

30. Ries, S.K. 1976. Subtoxic effects on plants, p. 313-344. In L.J. Audus (ed.) Herbicides, physiology, biochemistry, ecology, vol. 2. Academic Press, London.

31. Stevenson, J.C. and N.M. Confer. 1978. Summary of available information on Chesapeake Bay submerged vegetation. US Dept. Inter. FWS/OBS-78/66. NTIS, Springfield, VA. 333 pp.

32. Stevenson, J.C., W.R. Boynton, J.C. Means, W.M. Kemp, and R.R. Twilley. 1983. Concentrations of the herbicides, atrazine and linuron, in agricultural drainage, tributary estuaries and littoral zones of upper Chesapeake Bay. Report to US EPA, Annapolis, MD.

33. Streibig, J.C. Models for curve-fitting herbicides dose response data. Acta Agric. Scandinav. 30:59-64.

34. Strickland, J.D.H. and T.R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Fish. Res. Bd., Canada Bull. 167. Ottawa. 310 pp.

35. Sutton, D.L., D.A. Durham, S.W. Bingham, C.L. Foy. 1969. Influence of simazine on apparent photosynthesis of aquatic plants and herbicide residue removal from water. Weed. Sci. 17:56-59.

36. Vallentine, J.P. and S.W. Bingham. 1976. Influence of algae on amitrole and atrzine residues in water. J. Can. Bot. 54:2100-2107.

37. Walsh, G.E. 1972. Effect of herbicides on photosynthesis and growth of marine unicellular algae. Hyacinth. Contr. J. 10:45-48.

38. Walsh, G.D., D.L. Hansen and D.A. Lawrence. 1982. A flow-through system for exposure of seagrass to pollutants. Mar. Environ. Res. 7:1-11.

39. Wu, T.L. 1980. Dissipation of the herbicides atrazine and alachlor in a Maryland Cornfield. J. Environ. Qual. 9:459-465.

40. Zahnow, E.W. and J.D. Riggleman. 1980. Search for linuron residues in tributaries of the Chesapeake Bay.J. Agric. Food Chem. 28:974-978.

APPENDIX

TABLE 1A RESPONSE OF APPARENT PHOTOSYNTHESIS OF POTAMOGETON TO ATRAZINE CONCENTRATIONS(P.P.B.). 1979 EXPERIMENTS
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

			WEEK								
TREATMENT		TANK	3	4	5	6	7	8	9	10	11
CONTROL		11	.198	.165	.396	.403	.352	.603	.456	.527	.511
CONTROL		11	.244	.173	.392	.283	.591	.643	.548	.535	.541
CONTROL		22	.371	.390	.303	.260	.264	.653	.597	.593	.515
CONTROL		22	.428	.319	.338	.249	.572	.701	.514	.569	.629
MEAN			.310	.262	.357	.299	.445	.650	.529	.556	.549
ERROR			.107	.111	.045	.071	.162	.040	.059	.031	.055
VEH-CONTROL		19	.267	.142	.307	.247	.368	.480	.259	.600	.680
VEH-CONTROL		19	.293	.119	.313	.181	.546	.539	.237	.593	.737
VEH-CONTROL		20	.144	.259	.358	.429	.581	.530	.375	.516	.499
VEH-CONTROL		20	.253	.199	.380	.363	.641	.595	.333	.561	.525
MEAN			.239	.180	.339	.305	.534	.536	.301	.567	.610
ERROR			.066	.063	.035	.112	.117	.047	.064	.038	.116
ATRAZINE	5	12	.188	.203	.270	.322	.396	.453	.175	.486	.565
ATRAZINE	5	12	.246	.103	.239	.239	.404	.531	.142	.546	.618
ATRAZINE	5	13	.145	.143	.279	.453	.437	.491	.202	.285	.408
ATRAZINE	5	13	.187	.164	.316	.251	.426	.545	.111	.348	.449
MEAN			.191	.153	.276	.316	.416	.505	.157	.416	.510
ERROR			.041	.042	.032	.098	.019	.042	.040	.121	.098
ATRAZINE	50	3	.131	.130	.305	.307	.315	.221	-.145	.293	.339
ATRAZINE	50	3	.229	.130	.292	.197	.483	.342	-.077	.315	.346
ATRAZINE	50	24	.167	.297	.557	.543	.623	.235	-.017	.306	.331
ATRAZINE	50	24	.242	.233	.568	.506	.639	.339	-.039	.314	.348
MEAN			.192	.197	.430	.388	.515	.284	-.069	.307	.341
ERROR			.052	.082	.153	.164	.151	.065	.056	.010	.008
ATRAZINE	100	15	.206	.092	.297	.164	.129	-.053	-.372	.004	.125
ATRAZINE	100	15	.216	.119	.298	.049	.171	.045	-.499	.081	.158
ATRAZINE	100	27	.194	.206	.297	.410	.380	-.032	-.303	.075	.105
ATRAZINE	100	27	.282	.187	.295	.221	.431	.072	-.189	.105	.173
MEAN			.224	.151	.297	.211	.278	.008	-.341	.066	.140
ERROR			.039	.054	.001	.151	.150	.060	.130	.043	.031
ATRAZINE	500	16	.272	.270	.313	.260	.440	-.335	-.593	-.342	-.218
ATRAZINE	500	16	.392	.200	.288	.218	.558	-.153	-.491	-.298	-.155
ATRAZINE	500	26	.739	.201	.440	.359	.391	-.401	-.494	-.355	-.212
ATRAZINE	500	26	.085	.109	.423	.338	.564	-.253	-.489	-.395	-.208
MEAN			.372	.195	.366	.294	.488	-.285	-.517	-.347	-.198
ERROR			.275	.066	.077	.066	.086	.107	.051	.040	.029
ATRAZINE	1000	5	.179	.130	.250	.371	.522	-.191	-.326	-.237	-.170
ATRAZINE	1000	5	.253	.207	.375	.196	.503	-.107	-.335	-.223	-.115
ATRAZINE	1000	10	.196	.187	.258	.375	.418	-.323	-.420	-.403	-.205
ATRAZINE	1000	10	.287	.150	.312	.256	.485	-.164	-.449	-.299	-.150
MEAN			.209	.184	.301	.289	.470	-.183	-.397	-.274	-.159
ERROR			.050	.038	.050	.085	.041	.101	.068	.069	.031

TABLE 1B RESPONSE OF RESPIRATION OF POTAMOGETON TO ATRAZINE CONCENTRATIONS
(P.P.B.). 1979 EXPERIMENT.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

			WEEK								
TREATMENT		TANK	3	4	5	6	7	8	9	10	11
CONTROL		11	-.244	-.275	-.369	-.382	-.361	-.677	-.539	-.503	-.577
CONTROL		11	-.287	-.271	-.376	-.331	-.443	-.677	-.560	-.527	-.633
CONTROL		22	-.340	-.400	-.318	-.340	-.401	-.774	-.647	-.632	-.674
CONTROL		22	-.419	-.375	-.373	-.321	-.492	-.774	-.552	-.641	-.694
MEAN			-.322	-.330	-.359	-.343	-.424	-.725	-.574	-.576	-.644
ERROR			.075	.067	.027	.027	.056	.056	.049	.071	.052
VEH-CONTROL		19	-.375	-.349	-.403	-.311	-.463	-.713	-.740	-.627	-.721
VEH-CONTROL		19	-.406	-.300	-.407	-.295	-.544	-.713	-.724	-.642	-.769
VEH-CONTROL		20	-.206	-.316	-.341	-.402	-.458	-.674	-.668	-.584	-.576
VEH-CONTROL		20	-.265	-.282	-.370	-.375	-.495	-.674	-.710	-.619	-.593
MEAN			-.313	-.312	-.380	-.346	-.490	-.693	-.710	-.618	-.665
ERROR			.094	.028	.031	.051	.040	.023	.031	.025	.095
ATRAZINE	5	12	-.298	-.384	-.364	-.377	-.408	-.703	-.643	-.592	-.613
ATRAZINE	5	12	-.331	-.321	-.371	-.366	-.418	-.703	-.682	-.595	-.641
ATRAZINE	5	13	-.234	-.288	-.325	-.451	-.430	-.620	-.587	-.403	-.466
ATRAZINE	5	13	-.272	-.286	-.367	-.319	-.419	-.620	-.563	-.503	-.521
MEAN			-.284	-.320	-.357	-.378	-.419	-.661	-.619	-.523	-.560
ERROR			.041	.046	.021	.055	.009	.048	.054	.091	.081
ATRAZINE	50	3	-.218	-.258	-.332	-.306	-.348	-.584	-.612	-.411	-.437
ATRAZINE	50	3	-.268	-.242	-.335	-.249	-.316	-.584	-.503	-.430	-.457
ATRAZINE	50	24	-.311	-.396	-.508	-.575	-.586	-.707	-.548	-.589	-.796
ATRAZINE	50	24	-.342	-.347	-.549	-.543	-.611	-.707	-.611	-.593	-.800
MEAN			-.285	-.311	-.431	-.418	-.465	-.645	-.568	-.506	-.622
ERROR			.054	.073	.114	.165	.155	.071	.053	.099	.203
ATRAZINE	100	15	-.277	-.214	-.303	-.153	-.161	-.463	-.454	-.154	-.205
ATRAZINE	100	15	-.294	-.224	-.313	-.088	-.179	-.463	-.536	-.204	-.194
ATRAZINE	100	27	-.264	-.350	-.349	-.406	-.386	-.485	-.542	-.405	-.488
ATRAZINE	100	27	-.343	-.313	-.371	-.317	-.401	-.485	-.728	-.392	-.474
MEAN			-.294	-.275	-.334	-.241	-.282	-.474	-.565	-.289	-.340
ERROR			.035	.067	.032	.146	.129	.013	.116	.128	.163
ATRAZINE	500	16	-.317	-.349	-.365	-.319	-.473	-.550	-.476	-.350	-.189
ATRAZINE	500	16	-.381	-.297	-.347	-.283	-.475	-.550	-.523	-.326	-.235
ATRAZINE	500	26	-.821	-.403	-.432	-.497	-.487	-.527	-.476	-.495	-.374
ATRAZINE	500	26	-.282	-.424	-.479	-.489	-.576	-.527	-.513	-.494	-.379
MEAN			-.450	-.368	-.406	-.397	-.503	-.538	-.497	-.416	-.294
ERROR			.251	.057	.061	.112	.049	.013	.025	.091	.097
ATRAZINE	1000	5	-.207	-.230	-.282	-.357	-.461	-.311	-.327	-.196	-.143
ATRAZINE	1000	5	-.261	-.255	-.375	-.267	-.472	-.309	-.346	-.225	-.132
ATRAZINE	1000	10	-.336	-.342	-.397	-.421	-.464	-.531	-.374	-.295	-.144
ATRAZINE	1000	10	-.371	-.303	-.402	-.384	-.496	-.531	-.425	-.263	-.143
MEAN			-.289	-.302	-.377	-.334	-.456	-.520	-.379	-.235	-.144
ERROR			.058	.049	.048	.069	.030	.183	.049	.038	.007

TABLE 1C RESPONSE OF GROSS PHOTOSYNTHESIS OF POTAMOGETON TO ATRAZINE
CONCENTRATIONS(P.P.B.). 1979 EXPERIMENT.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

			WEEK								
TREATMENT		TANK	3	4	5	6	7	8	9	10	11
CONTROL		11	.540	.550	.912	.937	.858	1.552	1.210	1.232	1.318
CONTROL		11	.646	.552	.918	.746	1.211	1.591	1.333	1.273	1.426
CONTROL		22	.847	.950	.748	.736	.826	1.736	1.503	1.477	1.458
CONTROL		22	1.015	.843	.861	.699	1.261	1.784	1.288	1.467	1.600
MEAN			.762	.724	.860	.780	1.039	1.666	1.333	1.362	1.450
ERROR			.211	.204	.079	.107	.229	.112	.124	.128	.116
VEH-CONTROL		19	.792	.630	.872	.683	1.017	1.478	1.295	1.479	1.690
VEH-CONTROL		19	.862	.538	.883	.595	1.308	1.537	1.250	1.491	1.813
VEH-CONTROL		20	.433	.701	.835	.992	1.222	1.473	1.310	1.334	1.306
VEH-CONTROL		20	.624	.595	.898	.887	1.333	1.538	1.327	1.429	1.355
MEAN			.678	.616	.872	.789	1.220	1.506	1.295	1.433	1.541
ERROR			.191	.068	.027	.182	.143	.036	.033	.071	.249
ATRAZINE	5	12	.605	.740	.780	.851	.967	1.438	1.075	1.315	1.422
ATRAZINE	5	12	.709	.552	.758	.751	.990	1.516	1.097	1.379	1.515
ATRAZINE	5	13	.473	.546	.734	1.085	1.039	1.360	1.023	.850	1.061
ATRAZINE	5	13	.567	.563	.829	.697	1.012	1.414	.899	1.051	1.178
MEAN			.588	.600	.775	.846	1.002	1.432	1.023	1.149	1.294
ERROR			.098	.093	.040	.172	.031	.065	.089	.245	.211
ATRAZINE	50	3	.436	.492	.769	.736	.802	1.038	.712	.869	.950
ATRAZINE	50	3	.604	.468	.761	.546	.925	1.159	.627	.918	.985
ATRAZINE	50	24	.602	.852	1.269	1.349	1.443	1.224	.750	1.130	1.446
ATRAZINE	50	24	.721	.718	1.336	1.267	1.494	1.328	.817	1.145	1.468
MEAN			.591	.632	1.034	.974	1.166	1.187	.726	1.015	1.212
ERROR			.117	.185	.312	.394	.354	.121	.079	.142	.283
ATRAZINE	100	15	.593	.392	.721	.379	.354	.595	.263	.219	.412
ATRAZINE	100	15	.628	.432	.737	.172	.421	.692	.251	.367	.429
ATRAZINE	100	27	.563	.696	.786	.978	.919	.647	.456	.642	.788
ATRAZINE	100	27	.762	.624	.814	.665	.992	.751	.830	.653	.836
MEAN			.636	.536	.764	.548	.671	.671	.450	.470	.616
ERROR			.088	.147	.043	.350	.330	.066	.270	.213	.227
ATRAZINE	500	16	.716	.758	.824	.706	1.102	.435	.073	.148	.046
ATRAZINE	500	16	.926	.615	.773	.614	1.222	.618	.240	.158	.174
ATRAZINE	500	26	1.888	.765	1.045	1.055	1.073	.338	.173	.337	.312
ATRAZINE	500	26	.481	.703	1.094	1.023	1.370	.485	.230	.296	.322
MEAN			1.003	.710	.934	.850	1.192	.469	.179	.235	.213
ERROR			.618	.069	.159	.222	.135	.117	.077	.096	.131
ATRAZINE	1000	5	.469	.452	.644	.871	1.168	.245	.131	.037	.031
ATRAZINE	1000	5	.619	.563	.899	.570	1.164	.325	.149	.092	.069
ATRAZINE	1000	10	.667	.666	.813	.965	1.068	.420	.103	.010	-.004
ATRAZINE	1000	10	.807	.574	.875	.794	1.180	.579	.146	.069	.050
MEAN			.613	.606	.828	.757	1.109	.544	.134	.054	.042
ERROR			.117	.095	.100	.171	.072	.270	.017	.034	.028

TABLE 1D RESPONSE OF P-R RATIO OF POTAMOGETON TO ATRAZINE CONCENTRATIONS (P.P.B.). 1979 EXPERIMENT.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

TREATMENT	TANK	WEEK 3	4	5	6	7	8	9	10	11
CONTROL	11	.811	.600	1.073	1.055	.975	.891	.846	1.048	.886
CONTROL	11	.850	.638	1.043	.855	1.334	.950	.979	1.015	.855
CONTROL	22	1.091	.975	.953	.765	.658	.844	.923	.938	.764
CONTROL	22	1.021	.851	.906	.776	1.163	.906	.931	.888	.906
MEAN		.943	.766	.994	.863	1.032	.898	.920	.972	.853
ERROR		.134	.178	.078	.134	.290	.044	.055	.073	.063
VEH-CONTROL	19	.712	.407	.762	.794	.795	.673	.350	.957	.943
VEH-CONTROL	19	.722	.397	.769	.614	1.004	.756	.327	.924	.958
VEH-CONTROL	20	.699	.820	1.050	1.067	1.269	.786	.561	.884	.866
VEH-CONTROL	20	.955	.706	1.027	.968	1.295	.883	.469	.906	.885
MEAN		.772	.582	.902	.861	1.091	.774	.427	.918	.913
ERROR		.122	.214	.158	.199	.237	.087	.109	.031	.044
ATRAZINE 5	12	.631	.529	.742	.854	.971	.644	.272	.821	.922
ATRAZINE 5	12	.743	.321	.644	.653	.967	.755	.208	.918	.964
ATRAZINE 5	13	.620	.497	.858	1.004	1.016	.792	.344	.707	.876
ATRAZINE 5	13	.688	.573	.861	.787	1.017	.879	.197	.692	.862
MEAN		.670	.480	.776	.824	.993	.767	.255	.784	.906
ERROR		.057	.110	.104	.146	.027	.097	.068	.106	.046
ATRAZINE 50	3	.601	.504	.919	1.003	.905	.378	-.237	.713	.776
ATRAZINE 50	3	.854	.537	.872	.791	1.528	.586	-.153	.733	.757
ATRAZINE 50	24	.537	.750	1.096	.944	1.063	.332	-.031	.520	.416
ATRAZINE 50	24	.708	.671	1.035	.932	1.046	.479	-.064	.530	.435
MEAN		.675	.615	.980	.917	1.135	.444	-.121	.624	.596
ERROR		.139	.115	.103	.090	.271	.113	.093	.115	.197
ATRAZINE 100	15	.744	.430	.980	1.072	.801	-.114	-.819	.026	.610
ATRAZINE 100	15	.735	.531	.952	.557	.955	.097	-.931	.397	.814
ATRAZINE 100	27	.735	.589	.851	1.010	.984	-.066	-.559	.185	.215
ATRAZINE 100	27	.822	.597	.795	.697	1.075	.148	-.260	.268	.365
MEAN		.759	.537	.894	.834	.954	.016	-.642	.219	.501
ERROR		.042	.077	.086	.247	.114	.126	.299	.155	.265
ATRAZINE 500	16	.858	.774	.858	.815	.930	-.609	-1.246	-.977	-1.153
ATRAZINE 500	16	1.029	.673	.830	.770	1.175	-.278	-.939	-.914	-.660
ATRAZINE 500	26	.900	.499	1.019	.722	.803	-.761	-1.038	-.717	-.567
ATRAZINE 500	26	.301	.257	.883	.691	.979	-.480	-.953	-.800	-.549
MEAN		.772	.551	.897	.749	.972	-.532	-1.044	-.852	-.732
ERROR		.322	.226	.084	.054	.154	.205	.142	.116	.285
ATRAZINE 1000	5	.865	.565	.887	1.039	1.132	-.614	-.997	-1.209	-1.189
ATRAZINE 1000	5	.969	.812	1.000	.734	1.066	-.346	-.968	-.991	-.871
ATRAZINE 1000	10	.583	.547	.650	.891	.901	-.608	-1.123	-1.366	-1.424
ATRAZINE 1000	10	.774	.495	.776	.667	.978	-.309	-1.056	-1.137	-1.049
MEAN		.733	.616	.804	.857	1.032	-.385	-1.042	-1.162	-1.103
ERROR		.162	.116	.128	.153	.081	.206	.059	.145	.198

Table 2A. Summary of plant biomass (gdw/microcosm) for 2 macrophytic species exposed to several concentrations of atrazine. Numbers in parentheses designate specific microcosms.

Species	Date	Treatments (ppb)						
		Control	Vehicle Control	5	50	100	500	1000
P. perfoliatus	10/7/79 (Week 0)	0.60(11)* 0.71(18) 0.61(22) 0.65(28)	0.47(19) 0.67(20)	0.52(12) 0.54(13)	0.64(3) 0.77(24)	0.57(15) 0.70(27)	0.60(16) 0.60(26)	0.76(5) 0.76(10) 0.62(17)
	$\bar{x}$ ± S.D.	0.64 ± 0.05	0.57	0.53	0.71	0.64	0.60	0.71 ± 0.08
	15/8/79 (Week 4)	2.83 2.04 2.79 2.41	2.98 4.16	1.61 2.18	2.56 5.42	1.74 3.17	2.78 4.30	3.29 3.34 2.59
	$\bar{x}$ ± S.D.	2.51 ± 0.37	3.57	1.90	3.99	2.46	3.54	3.07 ± 0.42
	6/9/79 (Week 7)	9.38 4.30 9.53 6.26	10.33 15.02	7.54 9.77	7.40 15.64	3.21 7.80	4.19 4.01	6.72 4.67 3.58
	$\bar{x}$ ± S.D.	7.37 ± 2.54	12.68	8.66	11.52	5.51	4.10	4.99 ± 1.59
	21/9/79 (Week 9)	8.96 4.74 9.12 8.30	14.35 20.93	13.61 13.32	8.66 9.20	3.38 5.26	0.01 0.02	0.00 0.00 0.00
	$\bar{x}$ ± S.D.	7.78 ± 2.06	17.64	13.47	8.93	4.32	0.02	0.00 ± 0.00

Table 2A. Continued

Species	Date	Control	Vehicle Control	Treatments (ppb) 5	50	100	500	1000
M. spicatum	16/7/79 (Week 0)		0.49(37)	0.49(35)	0.46(31)	0.50	0.47(41)	0.40(32)
			0.46(39)	0.49(40)	0.47(36)	0.49(34)	0.41(42)	0.48(39)
		$\bar{x}$ =	0.48	0.49	0.47	0.50	0.44	0.44
	15/8/79 (Week 4)		2.09	2.49	3.38	2.41	1.57	2.50
			1.75	2.04	1.24	1.47	1.57	1.31
		$\bar{x}$ =	1.92	2.27	2.31	1.94	1.57	1.91
	11/9/79 (Week 8)		4.03	4.46	6.24	6.39	2.45	4.16
			4.36	4.64	3.16	3.85	2.09	2.53
		$\bar{x}$ =	4.19	4.55	4.70	5.12	2.27	3.35
	3/10/79 (Week 11)		8.99	5.56	8.30	9.94	0.78	0.00
			7.36	5.29	4.73	4.49	0.38	0.00
		$\bar{x}$ =	8.18	5.43	6.52	7.22	0.58	0.00

Table 3A. Summary of initial and final biomass (gdw/microcosm) for 2 macrophytic species exposed to the herbicide linuron. Numbers in parentheses designate specific microcosm.

Species	Date	Treatments (ppb)						
		Control	Vehicle Control	5	50	100	500	1000
P. perfoliatus	20/5/80 (Week 1)	0.80(19)*	0.90(24)	0.93(17)	0.88(1)	0.93(2)	0.87(7)	0.88(20)
		0.88(16)	0.92(22)	0.91(18)	0.84(4)	0.84(8)	0.83(2)	0.87(13)
		0.90(15)						
		0.88(26)						
	$\bar{x}$	0.87	0.91	0.92	0.87	0.89	0.85	0.88
	S.D.	± 0.04						
	30/7/80 (Week 12)	7.79	11.00	6.65	3.18	3.47	0.00	0.00
		7.22	13.17	13.53	3.85	2.46	0.00	0.00
		6.03						
		12.37						
	$\bar{x}$	8.35	12.09	10.09	3.52	2.97	0.00	0.00
	S.D.	± 2.78						
M. spicatum	12/5/80 (Week 0)	0.31(32)	0.31(29)	0.33(31)	0.32(37)	0.33(420	0.31(39)	0.33(34)
		0.32(36)	0.32(30)	0.30(41)	0.33(40)	0.32(38)	0.31(33)	0.31(35)
	$\bar{x}$	0.32	0.32	0.32	0.32	0.33	0.31	0.32
	30/7/80 (Week 12)	4.22	8.62	12.84	10.25	1.36	1.63	0.20
		8.26	8.45	7.78	5.40	5.66	0.86	1.65
	$\bar{x}$	6.24	8.54	10.31	7.83	3.51	1.25	0.93

*Numbers in parentheses designate specific microcosms.

TABLE 4A RESPONSE OF APPARENT PHOTOSYNTHESIS OF POTAMOGETON TO LINURON CONCENTRATIONS(P.P.B.). 1980 EXPERIMENT.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

							WEEK				
TREATMENT		TANK	3	4	5	6	7	8	9	10	11
CONTROL		19	.183	.027	.260	.140	.173	.210	.226	.207	.408
CONTROL		19	.169	.015	.257	.183	.081	.199	.238	.226	.382
CONTROL		6	.212	.089	.191	.260	.243	.301	.348	.378	.378
CONTROL		6	.164	.095	.238	.290	.179	.295	.353	.404	.382
CONTROL		15	.112	.030	.154	.123	.225	.227	.295	.271	.276
CONTROL		15	.092	.039	.156	.165	.163	.293	.307	.246	.286
CONTROL		26	.176	.024	.234	.377	.428	.602	.592	.630	.577
CONTROL		26	.161	-.037	.233	.413	.420	.607	.606	.608	.607
MEAN			.159	.035	.215	.244	.239	.342	.371	.371	.412
ERROR			.039	.042	.043	.110	.124	.167	.148	.168	.121
VEH-CONTROL		22	.319	.047	.282	.324	.299	.246	.457	.531	.517
VEH-CONTROL		22	.314	.047	.285	.336	.072	.371	.473	.527	.537
VEH-CONTROL		24	.266	.129	.340	.411	.348	.247	.440	.451	.497
VEH-CONTROL		24	.257	.134	.308	.418	.154	.377	.441	.445	.524
MEAN			.289	.089	.304	.372	.218	.310	.453	.488	.519
ERROR			.032	.049	.027	.049	.128	.074	.016	.047	.017
LINURON	5	17	.225	.003	.189	.212	.104	-.145	.318	.405	.319
LINURON	5	17	.218	.016	.170	.201	.037	-.005	.347	.406	.336
LINURON	5	18	.236	.087	.235	.273	.213	.318	.438	.460	.419
LINURON	5	18	.240	.072	.266	.330	.134	.360	.443	.452	.489
MEAN			.230	.044	.215	.254	.122	.132	.386	.431	.391
ERROR			.010	.041	.044	.060	.073	.246	.064	.029	.079
LINURON	50	1	.237	.043	.189	.183	-.042	-.136	.196	.170	.177
LINURON	50	1	.241	.145	.229	.187	-.123	.012	.198	.168	.194
LINURON	50	4	.233	.035	.127	.232	-.096	-.205	.120	.238	.310
LINURON	50	4	.163	.043	.122	.242	-.182	-.032	.147	.190	.285
MEAN			.218	.066	.167	.211	-.111	-.090	.165	.191	.241
ERROR			.037	.052	.051	.030	.058	.099	.038	.033	.066
LINURON	100	8	.330	.105	.166	.235	-.189	-.273	.098	.132	.162
LINURON	100	8	.292	.128	.208	.271	-.255	-.110	.115	.107	.206
LINURON	100	21	.198	.063	.247	.175	-.213	-.324	.014	.091	.134
LINURON	100	21	.137	.080	.213	.224	-.314	-.099	.066	.094	.153
MEAN			.239	.094	.208	.226	-.243	-.201	.073	.106	.164
ERROR			.088	.028	.033	.040	.055	.114	.044	.019	.030
LINURON	500	2	.180	.037	.152	.154	-.187	-.217	-.137	-.091	-.090
LINURON	500	2	.146	.047	.153	.168	-.243	-.168	-.146	-.104	-.061
LINURON	500	7	.309	.051	.192	.259	-.246	-.370	-.155	-.172	-.156
LINURON	500	7	.296	.083	.243	.267	-.379	-.255	-.141	-.173	-.136
MEAN			.233	.054	.185	.212	-.264	-.252	-.145	-.135	-.111
ERROR			.082	.020	.043	.059	.081	.086	.008	.044	.043
LINURON	1000	13	.170	.039	.076	.159	-.358	-.389	-.285	-.268	-.234
LINURON	1000	13	.172	.004	.085	.187	-.175	-.369	-.306	-.274	-.241
LINURON	1000	20	.183	.074	.293	.320	-.375	-.258	-.191	-.159	-.175
LINURON	1000	20	.149	.086	.256	.318	-.206	-.237	-.202	-.161	-.160
MEAN			.168	.051	.178	.246	-.278	-.313	-.246	-.215	-.202
ERROR			.014	.037	.113	.085	.103	.077	.058	.064	.041

TABLE 4B RESPONSE OF RESPIRATION OF POTAMOGETON TO LINURON CONCENTRATIONS (P.P.B.). 1980 EXPERIMENT
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

		WEEK								
TREATMENT	TANK	3	4	5	6	7	8	9	10	11
CONTROL	19	-.313	-.331	-.366	-.322	-.435	-.385	-.377	-.308	-.356
CONTROL	19	-.290	-.309	-.374	-.347	-.444	-.297	-.355	-.325	-.351
CONTROL	6	-.249	-.240	-.275	-.279	-.346	-.331	-.332	-.291	-.339
CONTROL	6	-.227	-.205	-.282	-.308	-.377	-.247	-.298	-.313	-.342
CONTROL	15	-.201	-.214	-.224	-.205	-.262	-.247	-.270	-.264	-.294
CONTROL	15	-.193	-.165	-.226	-.219	-.279	-.206	-.253	-.278	-.279
CONTROL	26	-.404	-.472	-.508	-.496	-.689	-.526	-.556	-.569	-.571
CONTROL	26	-.390	-.446	-.522	-.522	-.565	-.460	-.539	-.563	-.581
MEAN		-.283	-.298	-.347	-.337	-.425	-.337	-.372	-.364	-.389
ERROR		.081	.113	.118	.117	.145	.112	.116	.126	.119
VEH-CONTROL	22	-.352	-.308	-.400	-.403	-.661	-.551	-.544	-.486	-.486
VEH-CONTROL	22	-.354	-.320	-.410	-.459	-.752	-.483	-.520	-.499	-.479
VEH-CONTROL	24	-.357	-.360	-.426	-.429	-.594	-.454	-.423	-.408	-.415
VEH-CONTROL	24	-.350	-.355	-.433	-.464	-.683	-.379	-.417	-.427	-.393
MEAN		-.353	-.336	-.417	-.439	-.672	-.467	-.476	-.455	-.443
ERROR		.003	.026	.015	.028	.065	.071	.065	.044	.046
LINURON 5	17	-.287	-.309	-.330	-.330	-.447	-.570	-.346	-.263	-.368
LINURON 5	17	-.260	-.290	-.330	-.360	-.557	-.376	-.346	-.307	-.367
LINURON 5	18	-.348	-.321	-.358	-.390	-.616	-.452	-.500	-.454	-.478
LINURON 5	18	-.331	-.307	-.354	-.443	-.611	-.410	-.485	-.480	-.495
MEAN		-.306	-.307	-.343	-.381	-.558	-.452	-.419	-.376	-.427
ERROR		.040	.013	.015	.048	.079	.085	.085	.107	.069
LINURON 50	1	-.312	-.245	-.328	-.296	-.348	-.248	-.300	-.274	-.350
LINURON 50	1	-.336	-.317	-.334	-.336	-.387	-.218	-.290	-.290	-.344
LINURON 50	4	-.301	-.287	-.253	-.285	-.309	-.377	-.244	-.268	-.336
LINURON 50	4	-.235	-.262	-.246	-.303	-.378	-.187	-.231	-.282	-.294
MEAN		-.296	-.278	-.290	-.305	-.355	-.257	-.266	-.278	-.331
ERROR		.043	.031	.047	.022	.035	.083	.034	.010	.025
LINURON 100	8	-.248	-.250	-.262	-.260	-.316	-.372	-.223	-.217	-.221
LINURON 100	8	-.232	-.234	-.258	-.306	-.395	-.255	-.212	-.246	-.225
LINURON 100	21	-.256	-.252	-.291	-.268	-.430	-.314	-.251	-.298	-.296
LINURON 100	21	-.230	-.221	-.280	-.281	-.399	-.237	-.253	-.315	-.285
MEAN		-.241	-.239	-.273	-.279	-.385	-.294	-.235	-.269	-.257
ERROR		.013	.015	.015	.020	.049	.061	.020	.045	.039
LINURON 500	2	-.242	-.188	-.214	-.207	-.252	-.187	-.168	-.111	-.103
LINURON 500	2	-.218	-.172	-.235	-.230	-.218	-.163	-.159	-.133	-.101
LINURON 500	7	-.332	-.314	-.322	-.317	-.371	-.260	-.176	-.180	-.207
LINURON 500	7	-.313	-.280	-.325	-.330	-.362	-.232	-.145	-.203	-.177
MEAN		-.276	-.238	-.274	-.271	-.301	-.211	-.162	-.157	-.147
ERROR		.055	.069	.058	.062	.077	.044	.013	.042	.053
LINURON 1000	13	-.317	-.372	-.335	-.297	-.522	-.376	-.314	-.269	-.248
LINURON 1000	13	-.317	-.208	-.306	-.299	-.522	-.337	-.308	-.281	-.257
LINURON 1000	20	-.319	-.326	-.355	-.339	-.451	-.261	-.208	-.173	-.204
LINURON 1000	20	-.307	-.307	-.355	-.395	-.451	-.214	-.195	-.189	-.191
MEAN		-.315	-.303	-.338	-.332	-.486	-.297	-.256	-.228	-.225
ERROR		.005	.069	.023	.046	.041	.073	.063	.055	.032

TABLE 4C RESPONSE OF GROSS PHOTOSYNTHESIS OF POTAMOGETON TO LINURON
CONCENTRATIONS(P.P.B.). 1980 EXPERIMENT.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

TREATMENT		TANK	WEEK								
			3	4	5	6	7	8	9	10	11
CONTROL		19	.622	.491	.772	.591	.782	.749	.754	.638	.906
CONTROL		19	.575	.447	.781	.669	.703	.615	.735	.681	.874
CONTROL		6	.561	.425	.575	.651	.728	.764	.813	.785	.852
CONTROL		6	.482	.382	.632	.721	.707	.641	.769	.842	.861
CONTROL		15	.393	.330	.468	.410	.592	.572	.673	.641	.687
CONTROL		15	.362	.270	.472	.472	.554	.581	.661	.635	.677
CONTROL		26	.742	.686	.945	1.071	1.393	1.338	1.370	1.427	1.376
CONTROL		26	.707	.588	.964	1.144	1.212	1.251	1.361	1.395	1.420
MEAN			.555	.452	.701	.716	.834	.814	.892	.880	.957
ERROR			.137	.135	.195	.263	.302	.306	.296	.336	.286
VEH-CONTROL		22	.812	.479	.842	.889	1.224	1.017	1.219	1.212	1.197
VEH-CONTROL		22	.809	.496	.858	.978	1.124	1.048	1.201	1.226	1.207
VEH-CONTROL		24	.765	.634	.937	1.012	1.180	.883	1.032	1.022	1.078
VEH-CONTROL		24	.747	.630	.914	1.068	1.109	.907	1.024	1.043	1.075
MEAN			.783	.560	.888	.987	1.159	.964	1.119	1.126	1.139
ERROR			.032	.084	.045	.075	.053	.081	.105	.108	.073
LINURON	5	17	.626	.436	.651	.674	.729	.654	.803	.774	.833
LINURON	5	17	.581	.421	.632	.705	.816	.521	.832	.836	.849
LINURON	5	18	.723	.536	.736	.819	1.075	.951	1.137	1.095	1.088
LINURON	5	18	.703	.502	.762	.951	.990	.934	1.122	1.123	1.182
MEAN			.658	.474	.695	.787	.902	.765	.973	.957	.988
ERROR			.066	.054	.063	.126	.158	.212	.181	.178	.174
LINURON	50	1	.674	.387	.648	.598	.446	.211	.616	.554	.666
LINURON	50	1	.711	.590	.696	.658	.418	.316	.604	.574	.676
LINURON	50	4	.654	.438	.482	.631	.336	.323	.461	.613	.780
LINURON	50	4	.492	.409	.467	.666	.347	.230	.470	.585	.697
MEAN			.633	.456	.573	.638	.387	.270	.538	.581	.705
ERROR			.097	.092	.116	.031	.054	.058	.084	.025	.052
LINURON	100	8	.677	.456	.533	.598	.252	.248	.411	.436	.471
LINURON	100	8	.617	.455	.569	.699	.298	.247	.413	.450	.522
LINURON	100	21	.557	.415	.654	.550	.388	.116	.366	.508	.548
LINURON	100	21	.460	.390	.606	.618	.245	.233	.421	.536	.552
MEAN			.578	.429	.590	.616	.296	.211	.403	.482	.523
ERROR			.093	.032	.052	.062	.066	.064	.025	.047	.037
LINURON	500	2	.518	.300	.451	.444	.166	.045	.098	.064	.054
LINURON	500	2	.452	.288	.482	.490	.062	.060	.077	.083	.079
LINURON	500	7	.774	.490	.643	.703	.274	-.005	.091	.079	.134
LINURON	500	7	.733	.474	.698	.729	.128	.070	.062	.112	.113
MEAN			.619	.388	.568	.591	.157	.042	.082	.084	.095
ERROR			.158	.109	.121	.145	.089	.033	.016	.020	.036
LINURON	1000	13	.614	.560	.545	.575	.373	.137	.154	.109	.113
LINURON	1000	13	.617	.294	.514	.605	.555	.103	.125	.119	.119
LINURON	1000	20	.630	.530	.790	.796	.257	.106	.101	.084	.111
LINURON	1000	20	.578	.515	.752	.871	.426	.062	.071	.104	.108
MEAN			.610	.475	.650	.712	.403	.102	.113	.104	.113
ERROR			.022	.122	.141	.144	.124	.031	.035	.015	.005

TABLE 4D RESPONSE OF P-R RATIO OF POTAMOGETON TO LINURON CONCENTRATIONS (P.P.B.). 1980 EXPERIMENT.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

TREATMENT	TANK	WEEK 3	4	5	6	7	8	9	10	11
CONTROL	19	.585	.082	.710	.435	.398	.545	.599	.672	1.146
CONTROL	19	.583	.049	.687	.527	.182	.670	.670	.695	1.088
CONTROL	6	.851	.371	.695	.932	.702	.909	1.048	1.299	1.115
CONTROL	6	.722	.463	.844	.942	.475	1.194	1.185	1.291	1.117
CONTROL	15	.557	.140	.687	.600	.859	.919	1.093	1.027	.939
CONTROL	15	.477	.236	.690	.753	.584	1.422	1.213	.885	1.025
CONTROL	26	.436	.051	.461	.760	.621	1.144	1.065	1.107	1.011
CONTROL	26	.413	-.083	.446	.791	.743	1.320	1.124	1.080	1.045
MEAN		.578	.164	.652	.717	.570	1.015	1.000	1.007	1.061
ERROR		.148	.182	.134	.183	.215	.309	.233	.241	.069
VEH-CONTROL	22	.906	.153	.705	.804	.452	.446	.840	1.093	1.064
VEH-CONTROL	22	.887	.147	.695	.732	.096	.768	.910	1.056	1.121
VEH-CONTROL	24	.745	.358	.798	.958	.586	.544	1.040	1.105	1.198
VEH-CONTROL	24	.734	.377	.711	.901	.225	.995	1.058	1.042	1.333
MEAN		.818	.259	.727	.849	.340	.688	.962	1.074	1.179
ERROR		.091	.126	.048	.100	.220	.245	.105	.030	.116
LINURON 5	17	.784	.010	.573	.642	.233	-.254	.919	1.540	.867
LINURON 5	17	.838	.055	.515	.558	.066	-.013	1.003	1.322	.916
LINURON 5	18	.678	.271	.656	.700	.346	.704	.876	1.013	.877
LINURON 5	18	.725	.235	.751	.745	.219	.878	.913	.942	.988
MEAN		.756	.143	.624	.661	.216	.329	.928	1.204	.912
ERROR		.070	.129	.103	.081	.115	.547	.054	.278	.055
LINURON 50	1	.760	.176	.576	.618	-.121	-.548	.653	.620	.506
LINURON 50	1	.717	.457	.686	.557	-.318	.055	.683	.579	.564
LINURON 50	4	.774	.122	.502	.814	-.311	-.544	.492	.888	.923
LINURON 50	4	.694	.164	.496	.799	-.481	-.171	.636	.674	.969
MEAN		.736	.230	.565	.697	-.308	-.302	.616	.690	.740
ERROR		.037	.153	.088	.129	.147	.296	.085	.137	.239
LINURON 100	8	1.331	.420	.634	.904	-.598	-.734	.439	.608	.733
LINURON 100	8	1.259	.547	.806	.886	-.646	-.431	.542	.435	.916
LINURON 100	21	.773	.250	.849	.653	-.495	-1.032	.056	.305	.453
LINURON 100	21	.596	.362	.761	.797	-.787	-.418	.261	.298	.537
MEAN		.990	.395	.762	.810	-.631	-.654	.324	.411	.660
ERROR		.361	.124	.093	.115	.121	.291	.213	.145	.207
LINURON 500	2	.744	.197	.710	.744	-.742	-1.160	-.815	-.820	-.874
LINURON 500	2	.670	.273	.651	.730	-1.115	-1.031	-.918	-.782	-.604
LINURON 500	7	.931	.162	.596	.817	-.663	-1.423	-.881	-.956	-.754
LINURON 500	7	.946	.296	.748	.809	-1.047	-1.099	-.972	-.852	-.768
MEAN		.823	.232	.676	.775	-.892	-1.178	-.896	-.852	-.750
ERROR		.137	.063	.067	.044	.223	.171	.066	.075	.111
LINURON 1000	13	.536	.105	.227	.535	-.686	-1.035	-.908	-.996	-.944
LINURON 1000	13	.543	.019	.278	.625	-.335	-1.095	-.994	-.975	-.938
LINURON 1000	20	.574	.227	.825	.944	-.831	-.989	-.918	-.919	-.858
LINURON 1000	20	.485	.280	.721	.805	-.457	-1.107	-1.036	-.852	-.838
MEAN		.534	.158	.513	.727	-.577	-1.056	-.964	-.935	-.894
ERROR		.037	.118	.304	.183	.223	.055	.061	.064	.054

TABLE 5A RESPONSE OF APPARENT PHOTOSYNTHESIS OF MYRIOPHYLLUM TO ATRAZINE
CONCENTRATIONS(P.P.B.). 1979 EXPERIMENT
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

TREATMENT		TANK	WEEK 3	4	5	6	7	8	9	10	11
VEH-CONTROL		37	.134	.135	.454	.423	.446	.440	.229	.473	.450
VEH-CONTROL		37	.222	.095	.454	.264	.462	.489	.207	.440	.671
VEH-CONTROL		39	.216	.227	.445	.434	.437	.534	.516	.555	.539
VEH-CONTROL		39	.284	.239	.437	.272	.527	.591	.460	.547	.691
MEAN			.214	.174	.447	.348	.468	.513	.353	.504	.588
ERROR			.062	.070	.008	.093	.041	.064	.158	.056	.114
ATRAZINE	5	35	.188	.380	.473	.323	.351	.454	.264	.565	.548
ATRAZINE	5	35	.297	.325	.353	.126	.519	.503	.230	.542	.765
ATRAZINE	5	40	.456	.187	.546	.499	.546	.722	.638	.791	.640
ATRAZINE	5	40	.593	.230	.517	.292	.591	.788	.436	.798	.735
MEAN			.383	.280	.472	.310	.502	.617	.392	.674	.672
ERROR			.178	.088	.085	.153	.105	.163	.187	.139	.098
ATRAZINE	50	31	.200	.231	.514	.603	.568	.236	.087	.392	.391
ATRAZINE	50	31	.288	.091	.541	.248	.729	.326	.148	.344	.512
ATRAZINE	50	26	.000	.000	.000	.000	.000	.000	.000	.000	.000
ATRAZINE	50	26	.000	.000	.000	.000	.000	.000	.000	.000	.000
MEAN			.366	.349	.482	.424	.538	.211	-.134	.393	.514
ERROR			.151	.227	.054	.149	.146	.098	.313	.060	.097
ATRAZINE	100	33	.178	.340	.532	.461	.398	-.033	-.037	.265	.298
ATRAZINE	100	33	.357	.313	.518	.297	.480	.055	-.003	.314	.422
ATRAZINE	100	34	.239	.294	.400	.393	.383	-.133	-.483	.532	.268
ATRAZINE	100	34	.270	.215	.371	.286	.472	.015	-.488	.482	.303
MEAN			.261	.290	.455	.359	.433	-.024	-.253	.398	.323
ERROR			.075	.054	.082	.083	.050	.081	.269	.129	.068
ATRAZINE	500	41	.095	.103	.226	.181	.177	-.253	-.331	-.112	-.105
ATRAZINE	500	41	.310	.149	.210	.102	.222	-.156	-.235	-.103	-.133
ATRAZINE	500	42	.189	.138	.250	.249	.233	-.220	-.500	-.170	-.040
ATRAZINE	500	42	.222	.084	.229	.191	.294	-.103	-.488	-.104	-.055
MEAN			.204	.118	.229	.181	.231	-.183	-.388	-.122	-.083
ERROR			.089	.030	.016	.060	.048	.067	.128	.032	.043
ATRAZINE	1000	32	.408	.432	.457	.557	.629	-.561	-.707	-.321	-.376
ATRAZINE	1000	32	.432	.316	.463	.425	.758	-.342	-.491	-.319	-.379
ATRAZINE	1000	38	.320	.421	.377	.417	.321	-.517	-.501	-.271	-.272
ATRAZINE	1000	38	.523	.369	.399	.291	.422	-.373	-.490	-.160	-.255
MEAN			.421	.384	.424	.422	.532	-.448	-.547	-.268	-.320
ERROR			.083	.053	.043	.109	.198	.107	.107	.075	.066

TABLE 5B RESPONSE OF RESPIRATION OF MYRIOPHYLLUM TO ATRAZINE CONCENTRATIONS (P.P.B.). 1979 EXPERIMENT
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

TREATMENT	TANK	WEEK 3	4	5	6	7	8	9	10	11
VEH-CONTROL	37	-.185	-.166	-.445	-.436	-.443	-.660	-.691	-.576	-.593
VEH-CONTROL	37	-.301	-.125	-.494	-.360	-.464	-.660	-.684	-.542	-.621
VEH-CONTROL	39	-.269	-.310	-.443	-.446	-.456	-.695	-.548	-.588	-.589
VEH-CONTROL	39	-.351	-.316	-.436	-.343	-.493	-.695	-.601	-.585	-.611
MEAN		-.276	-.229	-.454	-.396	-.464	-.677	-.631	-.573	-.604
ERROR		.070	.098	.027	.052	.021	.020	.069	.021	.015
ATRAZINE 5	35	-.247	-.432	-.439	-.320	-.409	-.628	-.565	-.620	-.654
ATRAZINE 5	35	-.354	-.419	-.376	-.107	-.492	-.628	-.495	-.622	-.695
ATRAZINE 5	40	-.406	-.334	-.554	-.521	-.528	-.875	-1.118	-.798	-.682
ATRAZINE 5	40	-.526	-.343	-.579	-.447	-.574	-.875	-.833	-.774	-.632
MEAN		-.383	-.382	-.487	-.349	-.501	-.751	-.753	-.703	-.666
ERROR		.116	.051	.096	.181	.070	.143	.284	.096	.028
ATRAZINE 50	31	-.231	-.210	-.467	-.599	-.565	-.704	-.686	-.528	-.597
ATRAZINE 50	31	-.304	-.122	-.515	-.236	-.624	-.704	-.437	-.524	-.583
ATRAZINE 50	26	.000	.000	.000	.000	.000	.000	.000	.000	.000
ATRAZINE 50	26	.000	.000	.000	.000	.000	.000	.000	.000	.000
MEAN		-.447	-.395	-.567	-.518	-.599	-.736	-.553	-.547	-.610
ERROR		.222	.267	.091	.189	.030	.037	.102	.035	.024
ATRAZINE 100	33	-.339	-.443	-.501	-.488	-.469	-.493	-.539	-.434	-.515
ATRAZINE 100	33	-.461	-.434	-.533	-.401	-.498	-.493	-.776	-.498	-.515
ATRAZINE 100	34	-.437	-.487	-.507	-.516	-.471	-.663	-.491	-.624	-.561
ATRAZINE 100	34	-.490	-.462	-.511	-.451	-.500	-.663	-.514	-.615	-.537
MEAN		-.432	-.456	-.513	-.464	-.484	-.578	-.580	-.543	-.532
ERROR		.066	.023	.014	.050	.017	.098	.132	.092	.022
ATRAZINE 500	41	-.254	-.282	-.297	-.253	-.207	-.360	-.305	-.154	-.263
ATRAZINE 500	41	-.394	-.284	-.301	-.178	-.319	-.360	-.254	-.159	-.260
ATRAZINE 500	42	-.319	-.347	-.333	-.339	-.315	-.448	-.478	-.244	-.327
ATRAZINE 500	42	-.407	-.335	-.351	-.279	-.332	-.448	-.515	-.217	-.302
MEAN		-.343	-.312	-.320	-.262	-.293	-.404	-.388	-.193	-.288
ERROR		.071	.034	.026	.067	.058	.051	.128	.044	.032
ATRAZINE 1000	32	-.374	-.534	-.515	-.579	-.522	-.546	-.470	-.428	-.497
ATRAZINE 1000	32	-.483	-.495	-.512	-.527	-.591	-.546	-.514	-.559	-.387
ATRAZINE 1000	38	-.434	-.476	-.417	-.565	-.393	-.517	-.476	-.229	-.372
ATRAZINE 1000	38	-.572	-.489	-.480	-.402	-.431	-.517	-.512	-.247	-.337
MEAN		-.466	-.498	-.481	-.518	-.484	-.531	-.493	-.365	-.398
ERROR		.084	.025	.046	.081	.089	.017	.024	.157	.069

TABLE 5C RESPONSE OF GROSS PHOTOSYNTHESIS OF MYRIOPHYLLUM TO ATRAZINE CONCENTRATIONS(P.P.B.). 1979 EXPERIMENT
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

TREATMENT	TANK	WEEK 3	4	5	6	7	8	9	10	11
VEH-CONTROL	37	.393	.367	1.077	1.033	1.066	1.364	1.197	1.279	1.281
VEH-CONTROL	37	.644	.271	1.146	.768	1.111	1.413	1.166	1.199	1.541
VEH-CONTROL	39	.593	.661	1.064	1.058	1.075	1.507	1.284	1.378	1.364
VEH-CONTROL	39	.776	.682	1.047	.751	1.217	1.564	1.301	1.366	1.546
MEAN		.601	.495	1.083	.902	1.117	1.462	1.237	1.305	1.433
ERROR		.159	.207	.043	.166	.069	.090	.066	.084	.132
ATRAZINE 5	35	.534	.985	1.088	.772	.924	1.333	1.055	1.432	1.464
ATRAZINE 5	35	.792	.912	.880	.275	1.208	1.383	.922	1.413	1.737
ATRAZINE 5	40	1.024	.654	1.321	1.228	1.285	1.947	2.203	1.909	1.594
ATRAZINE 5	40	1.329	.710	1.328	.917	1.395	2.013	1.602	1.882	1.620
MEAN		.920	.815	1.154	.798	1.203	1.669	1.446	1.659	1.604
ERROR		.338	.158	.214	.397	.201	.361	.584	.273	.112
ATRAZINE 50	31	.524	.525	1.168	1.442	1.360	1.222	1.046	1.131	1.226
ATRAZINE 50	31	.713	.262	1.261	.579	1.603	1.312	.760	1.078	1.329
ATRAZINE 50	26	.000	.000	.000	.000	.000	.000	.000	.000	.000
ATRAZINE 50	26	.000	.000	.000	.000	.000	.000	.000	.000	.000
MEAN		.992	.901	1.276	1.150	1.378	1.241	.640	1.160	1.368
ERROR		.460	.597	.082	.392	.164	.063	.344	.069	.118
ATRAZINE 100	33	.653	.960	1.233	1.144	1.055	.658	.717	.873	1.018
ATRAZINE 100	33	1.003	.920	1.265	.859	1.177	.746	1.083	1.011	1.143
ATRAZINE 100	34	.850	.976	1.110	1.115	1.043	.795	.205	1.406	1.053
ATRAZINE 100	34	.956	.861	1.086	.917	1.172	.942	.231	1.343	1.054
MEAN		.865	.929	1.173	1.009	1.112	.785	.559	1.158	1.067
ERROR		.155	.051	.089	.142	.073	.119	.421	.257	.053
ATRAZINE 500	41	.450	.497	.642	.535	.467	.251	.096	.104	.263
ATRAZINE 500	41	.861	.547	.632	.351	.669	.348	.121	.120	.232
ATRAZINE 500	42	.636	.623	.716	.724	.674	.407	.170	.171	.417
ATRAZINE 500	42	.792	.553	.720	.581	.758	.524	.233	.201	.367
MEAN		.685	.555	.677	.548	.642	.382	.155	.149	.320
ERROR		.183	.052	.047	.154	.124	.114	.060	.045	.087
ATRAZINE 1000	32	.932	1.179	1.178	1.367	1.361	.203	-.050	.277	.319
ATRAZINE 1000	32	1.108	1.009	1.180	1.162	1.585	.422	.229	.462	.162
ATRAZINE 1000	38	.927	1.087	.960	1.209	.871	.207	.166	.050	.249
ATRAZINE 1000	38	1.323	1.053	1.070	.854	1.025	.351	.228	.186	.217
MEAN		1.072	1.082	1.097	1.148	1.210	.296	.143	.244	.237
ERROR		.187	.072	.105	.215	.323	.109	.132	.173	.066

TABLE 5D RESPONSE OF P-R RATIO OF MYRIOPHYLLUM TO ATRAZINE CONCENTRATIONS
(P.P.B.). 1979 EXPERIMENT.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

TREATMENT	TANK	WEEK 3	4	5	6	7	8	9	10	11
VEH-CONTROL	37	.724	.813	1.020	.970	1.007	.667	.331	.821	.759
VEH-CONTROL	37	.738	.760	.919	.733	.996	.741	.303	.812	1.081
VEH-CONTROL	39	.803	.732	1.005	.973	.958	.768	.942	.944	.915
VEH-CONTROL	39	.809	.756	1.002	.793	1.069	.850	.765	.935	1.131
MEAN		.768	.765	.986	.867	1.007	.756	.585	.878	.971
ERROR		.044	.034	.046	.123	.046	.076	.318	.071	.169
ATRAZINE 5	35	.761	.880	1.077	1.009	.858	.723	.467	.911	.838
ATRAZINE 5	35	.839	.776	.939	1.178	1.055	.801	.465	.871	1.101
ATRAZINE 5	40	1.123	.560	.986	.958	1.034	.825	.571	.991	.938
ATRAZINE 5	40	1.127	.671	.893	.653	1.030	.901	.523	1.031	1.163
MEAN		.962	.722	.974	.949	.994	.812	.506	.951	1.010
ERROR		.190	.138	.079	.219	.091	.073	.051	.073	.149
ATRAZINE 50	31	.866	1.100	1.101	1.007	1.005	.335	.127	.742	.655
ATRAZINE 50	31	.947	.746	1.050	1.051	1.168	.463	.339	.656	.878
ATRAZINE 50	26	.000	.000	.000	.000	.000	.000	.000	.000	.000
ATRAZINE 50	26	.000	.000	.000	.000	.000	.000	.000	.000	.000
MEAN		.846	.892	.876	.855	.897	.291	-.237	.721	.841
ERROR		.082	.165	.231	.205	.230	.146	.588	.127	.145
ATRAZINE 100	33	.525	.767	1.062	.945	.849	-.067	-.069	.611	.579
ATRAZINE 100	33	.774	.721	.972	.741	.964	.112	-.004	.631	.819
ATRAZINE 100	34	.547	.604	.789	.762	.813	-.201	-.984	.853	.478
ATRAZINE 100	34	.551	.465	.726	.634	.944	.023	-.949	.784	.564
MEAN		.599	.639	.887	.770	.892	-.033	-.501	.720	.610
ERROR		.117	.135	.156	.129	.073	.134	.538	.118	.146
ATRAZINE 500	41	.374	.365	.761	.715	.855	-.703	-1.085	-.727	-.399
ATRAZINE 500	41	.787	.525	.698	.573	.696	-.433	-.925	-.648	-.512
ATRAZINE 500	42	.592	.398	.751	.735	.740	-.491	-1.046	-.697	-.122
ATRAZINE 500	42	.545	.251	.652	.685	.886	-.230	-.948	-.479	-.182
MEAN		.574	.385	.715	.677	.794	-.464	-1.001	-.638	-.304
ERROR		.170	.113	.051	.072	.091	.195	.077	.111	.183
ATRAZINE 1000	32	1.091	.809	.887	.962	1.205	-1.027	-1.504	-.750	-.757
ATRAZINE 1000	32	.894	.638	.904	.806	1.283	-.626	-.955	-.572	-.979
ATRAZINE 1000	38	.737	.884	.904	.738	.817	-1.000	-1.053	-1.183	-.731
ATRAZINE 1000	38	.914	.755	.831	.724	.979	-.721	-.955	-.648	-.757
MEAN		.909	.771	.881	.807	1.071	-.843	-1.117	-.788	-.806
ERROR		.145	.104	.035	.109	.213	.200	.262	.273	.116

TABLE 6A RESPONSE OF APPARENT PHOTOSYNTHESIS OF MYRIOPHYLLUM TO LINURON
CONCENTRATIONS(P.P.B.). 1980 EXPERIMENT.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

			WEEK								
TREATMENT		TANK	3	4	5	6	7	8	9	10	11
CONTROL		29	.398	.148	.101	.149	.276	.370	.360	.557	.511
CONTROL		29	.343	.103	.099	.196	.211	.311	.401	.530	.552
CONTROL		30	.262	.069	.116	.106	.166	.274	.354	.412	.431
CONTROL		30	.317	.003	.121	.199	.063	.246	.355	.419	.373
MEAN			.330	.081	.109	.162	.179	.300	.367	.479	.467
ERROR			.057	.061	.011	.044	.090	.054	.022	.075	.080
VEH-CONTROL		32	.322	-.020	.053	.152	.296	-.066	.339	.357	.402
VEH-CONTROL		32	.242	.011	.022	.238	.243	.133	.326	.352	.363
VEH-CONTROL		36	.393	.080	.193	.355	.347	.147	.409	.394	.389
VEH-CONTROL		36	.343	.072	.238	.318	.246	.270	.393	.405	.391
MEAN			.325	.036	.126	.266	.283	.121	.367	.377	.386
ERROR			.063	.048	.105	.090	.049	.139	.040	.026	.017
LINURON	5	31	.546	.049	.235	.323	.265	.391	.537	.601	.571
LINURON	5	31	.467	.038	.201	.319	.187	.430	.579	.583	.652
LINURON	5	41	.603	-.014	.136	.226	.139	-.123	.334	.467	.476
LINURON	5	41	.547	-.009	.096	.236	-.020	.039	.350	.436	.514
MEAN			.541	.016	.167	.276	.143	.184	.450	.522	.553
ERROR			.056	.032	.063	.052	.120	.270	.126	.082	.077
LINURON	50	37	.788	.146	.336	.275	-.113	.143	.446	.474	.473
LINURON	50	37	.724	.159	.257	.275	-.253	.200	.448	.481	.535
LINURON	50	40	.381	.120	.143	.322	-.008	.041	.214	.216	.277
LINURON	50	40	.340	.111	.149	.323	-.072	.049	.231	.202	.280
MEAN			.558	.134	.221	.299	-.111	.108	.335	.343	.391
ERROR			.230	.022	.093	.027	.104	.077	.130	.155	.133
LINURON	100	42	.273	.098	.125	.104	-.175	-.194	-.085	.014	.028
LINURON	100	42	.297	.060	.113	.148	-.367	-.168	-.062	.020	.010
LINURON	100	38	.275	.156	.381	.456	-.163	-.160	.054	.148	.198
LINURON	100	38	.270	.133	.407	.455	-.223	-.040	.082	.150	.193
MEAN			.279	.112	.256	.291	-.232	-.140	-.003	.083	.107
ERROR			.012	.042	.159	.191	.094	.069	.083	.076	.102
LINURON	500	39	.598	.071	.334	.367	-.455	-.329	-.250	-.249	-.228
LINURON	500	39	.464	.084	.275	.419	-.263	-.325	-.245	-.264	-.213
LINURON	500	33	.359	.103	.172	.324	-.287	-.415	-.171	-.148	-.125
LINURON	500	33	.295	.101	.172	.367	-.352	-.274	-.176	-.139	-.214
MEAN			.429	.090	.238	.369	-.339	-.336	-.210	-.200	-.195
ERROR			.132	.015	.080	.039	.086	.058	.043	.066	.047
LINURON	1000	34	.440	.003	.164	.185	-.347	-.275	-.244	-.226	-.239
LINURON	1000	34	.340	.023	.159	.277	-.205	-.273	-.258	-.237	-.229
LINURON	1000	35	.429	.136	.352	.475	-.533	-.297	-.266	-.259	-.266
LINURON	1000	35	.285	.152	.334	.492	-.333	-.282	-.253	-.252	-.249
MEAN			.373	.078	.252	.357	-.354	-.282	-.255	-.243	-.246
ERROR			.074	.076	.105	.151	.135	.011	.009	.015	.016

TABLE 6B RESPONSE OF RESPIRATION OF MYRIOPHYLLUM TO LINURON CONCENTRATIONS
(P.P.B.). 1980 EXPERIMENT.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR

TREATMENT		TANK	WEEK								
			3	4	5	6	7	8	9	10	11
CONTROL		29	-.288	-.332	-.294	-.278	-.324	-.329	-.306	-.239	-.315
CONTROL		29	-.249	-.307	-.270	-.289	-.363	-.235	-.286	-.269	-.302
CONTROL		30	-.294	-.326	-.316	-.310	-.428	-.310	-.276	-.239	-.323
CONTROL		30	-.274	-.311	-.312	-.327	-.358	-.242	-.245	-.272	-.291
MEAN			-.276	-.319	-.298	-.301	-.368	-.279	-.278	-.255	-.308
ERROR			.020	.012	.021	.022	.043	.047	.025	.018	.014
VEH-CONTROL		32	-.284	-.253	-.279	-.285	-.330	-.389	-.342	-.287	-.369
VEH-CONTROL		32	-.258	-.254	-.259	-.332	-.407	-.280	-.316	-.327	-.327
VEH-CONTROL		36	-.322	-.314	-.304	-.373	-.449	-.476	-.341	-.319	-.391
VEH-CONTROL		36	-.306	-.317	-.295	-.390	-.443	-.302	-.319	-.359	-.360
MEAN			-.293	-.284	-.284	-.345	-.407	-.362	-.329	-.323	-.362
ERROR			.028	.036	.020	.047	.055	.090	.014	.030	.027
LINURON	5	31	-.451	-.321	-.363	-.421	-.566	-.485	-.506	-.454	-.552
LINURON	5	31	-.415	-.291	-.340	-.441	-.609	-.420	-.484	-.471	-.543
LINURON	5	41	-.399	-.326	-.365	-.378	-.522	-.501	-.438	-.377	-.454
LINURON	5	41	-.380	-.301	-.345	-.400	-.620	-.413	-.426	-.406	-.433
MEAN			-.411	-.310	-.353	-.410	-.579	-.455	-.463	-.427	-.495
ERROR			.030	.017	.013	.027	.045	.045	.038	.043	.061
LINURON	50	37	-.550	-.485	-.565	-.527	-.441	-.474	-.580	-.538	-.568
LINURON	50	37	-.625	-.489	-.538	-.559	-.601	-.400	-.562	-.566	-.590
LINURON	50	40	-.288	-.290	-.299	-.325	-.242	-.205	-.295	-.301	-.345
LINURON	50	40	-.283	-.275	-.263	-.367	-.305	-.149	-.277	-.322	-.310
MEAN			-.436	-.385	-.416	-.444	-.397	-.307	-.428	-.432	-.453
ERROR			.177	.118	.157	.116	.159	.155	.165	.140	.146
LINURON	100	42	-.231	-.290	-.286	-.280	-.325	-.289	-.262	-.228	-.293
LINURON	100	42	-.227	-.281	-.274	-.326	-.497	-.231	-.259	-.252	-.278
LINURON	100	38	-.239	-.273	-.300	-.347	-.341	-.258	-.226	-.214	-.300
LINURON	100	38	-.235	-.248	-.303	-.384	-.341	-.193	-.223	-.255	-.272
MEAN			-.233	-.273	-.291	-.334	-.376	-.243	-.242	-.237	-.286
ERROR			.005	.018	.013	.043	.081	.041	.021	.020	.013
LINURON	500	39	-.411	-.377	-.406	-.447	-.522	-.416	-.352	-.324	-.368
LINURON	500	39	-.356	-.349	-.390	-.457	-.522	-.363	-.351	-.368	-.354
LINURON	500	33	-.286	-.257	-.280	-.298	-.339	-.302	-.219	-.165	-.161
LINURON	500	33	-.240	-.265	-.266	-.308	-.374	-.262	-.196	-.250	-.085
MEAN			-.323	-.312	-.335	-.377	-.439	-.336	-.279	-.277	-.242
ERROR			.075	.060	.073	.086	.097	.068	.084	.089	.141
LINURON	1000	34	-.338	-.347	-.331	-.346	-.399	-.293	-.273	-.216	-.238
LINURON	1000	34	-.307	-.365	-.314	-.378	-.399	-.237	-.264	-.247	-.238
LINURON	1000	35	-.350	-.423	-.456	-.476	-.493	-.322	-.291	-.242	-.250
LINURON	1000	35	-.375	-.343	-.441	-.514	-.493	-.277	-.282	-.245	-.247
MEAN			-.342	-.369	-.385	-.428	-.446	-.282	-.277	-.237	-.243
ERROR			.028	.037	.073	.079	.054	.035	.012	.014	.006

TABLE 6C RESPONSE OF GROSS PHOTOSYNTHESIS OF MYRIOPHYLLUM TO LINURON
CONCENTRATIONS(P.P.B.). 1980 EXPERIMNET.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

			WEEK								
TREATMENT		TANK	3	4	5	6	7	8	9	10	11
CONTROL		29	.801	.613	.512	.539	.730	.831	.789	.892	.952
CONTROL		29	.692	.534	.477	.600	.720	.641	.800	.906	.975
CONTROL		30	.674	.526	.558	.540	.765	.707	.740	.747	.884
CONTROL		30	.700	.439	.558	.657	.565	.584	.698	.800	.779
MEAN			.717	.528	.526	.584	.695	.691	.757	.836	.897
ERROR			.057	.071	.039	.056	.089	.106	.047	.076	.088
VEH-CONTROL		32	.720	.334	.443	.550	.758	.479	.819	.758	.919
VEH-CONTROL		32	.603	.366	.385	.702	.813	.525	.769	.810	.821
VEH-CONTROL		36	.844	.519	.619	.878	.975	.813	.886	.841	.937
VEH-CONTROL		36	.771	.517	.650	.864	.866	.693	.839	.907	.896
MEAN			.734	.434	.524	.748	.853	.627	.828	.829	.893
ERROR			.101	.098	.130	.155	.093	.154	.048	.062	.051
LINURON	5	31	1.178	.499	.744	.912	1.058	1.070	1.246	1.236	1.344
LINURON	5	31	1.048	.445	.677	.937	1.039	1.018	1.256	1.242	1.412
LINURON	5	41	1.162	.442	.646	.756	.869	.578	.947	.994	1.111
LINURON	5	41	1.079	.413	.579	.795	.848	.617	.946	1.004	1.120
MEAN			1.117	.450	.661	.850	.953	.821	1.099	1.119	1.247
ERROR			.063	.036	.069	.088	.110	.259	.176	.139	.154
LINURON	50	37	1.559	.826	1.128	1.013	.504	.806	1.258	1.227	1.268
LINURON	50	37	1.599	.843	1.011	1.058	.587	.760	1.236	1.273	1.361
LINURON	50	40	.784	.526	.562	.776	.331	.329	.627	.638	.759
LINURON	50	40	.737	.497	.517	.836	.356	.258	.618	.653	.714
MEAN			1.170	.673	.804	.921	.444	.538	.935	.948	1.025
ERROR			.473	.187	.310	.136	.122	.285	.361	.350	.336
LINURON	100	42	.596	.503	.526	.495	.280	.210	.282	.333	.439
LINURON	100	42	.615	.453	.497	.605	.329	.156	.301	.373	.399
LINURON	100	38	.610	.538	.802	.941	.314	.202	.370	.447	.618
LINURON	100	38	.600	.480	.831	.992	.255	.231	.394	.507	.575
MEAN			.605	.493	.664	.758	.294	.200	.337	.415	.508
ERROR			.009	.036	.177	.246	.033	.032	.054	.077	.105
LINURON	500	39	1.174	.599	.902	.994	.276	.254	.242	.205	.288
LINURON	500	39	.962	.572	.821	1.060	.468	.183	.247	.252	.282
LINURON	500	33	.759	.464	.565	.741	.188	.008	.135	.084	.101
LINURON	500	33	.631	.472	.544	.798	.172	.092	.098	.211	-.094
MEAN			.881	.527	.708	.898	.276	.134	.180	.188	.144
ERROR			.238	.069	.181	.153	.136	.107	.075	.072	.181
LINURON	1000	34	.913	.489	.627	.669	.211	.135	.138	.077	.095
LINURON	1000	34	.770	.534	.600	.807	.353	.059	.111	.108	.104
LINURON	1000	35	.919	.728	.990	1.142	.157	.154	.140	.080	.084
LINURON	1000	35	.811	.631	.952	1.211	.357	.106	.141	.091	.098
MEAN			.853	.595	.792	.957	.269	.113	.132	.089	.095
ERROR			.074	.106	.207	.261	.101	.041	.014	.014	.008

TABLE 6D RESPONSE OF P-R RATIO OF MYRIOPHYLLUM TO LINURON CONCENTRATIONS
(P.P.B.). 1980 EXPERIMENT.
UNITS ARE GRAMS OXYGEN PER CUBIC METER PER HOUR.

TREATMENT		TANK	WEEK								
			3	4	5	6	7	8	9	10	11
CONTROL		29	1.382	.446	.344	.536	.852	1.125	1.176	2.331	1.622
CONTROL		29	1.378	.336	.367	.678	.581	1.323	1.402	1.970	1.828
CONTROL		30	.891	.212	.367	.342	.388	.884	1.283	1.724	1.334
CONTROL		30	1.157	.010	.388	.609	.176	1.017	1.449	1.540	1.282
MEAN			1.202	.251	.366	.541	.499	1.087	1.327	1.891	1.516
ERROR			.232	.187	.018	.145	.288	.186	.123	.342	.256
VEH-CONTROL		32	1.134	-.079	.190	.533	.897	-.170	.991	1.244	1.089
VEH-CONTROL		32	.938	.043	.085	.717	.597	.475	1.032	1.076	1.110
VEH-CONTROL		36	1.220	.255	.635	.952	.773	.309	1.199	1.235	.995
VEH-CONTROL		36	1.121	.227	.807	.815	.555	.894	1.232	1.128	1.086
MEAN			1.103	.111	.429	.754	.705	.377	1.113	1.171	1.070
ERROR			.119	.158	.347	.176	.159	.440	.120	.082	.051
LINURON	5	31	1.211	.153	.647	.767	.468	.806	1.061	1.324	1.034
LINURON	5	31	1.125	.131	.591	.723	.307	1.024	1.196	1.238	1.201
LINURON	5	41	1.511	-.043	.373	.598	.266	-.246	.763	1.239	1.048
LINURON	5	41	1.439	-.030	.278	.590	-.032	.094	.822	1.074	1.187
MEAN			1.321	.053	.472	.669	.252	.420	.960	1.219	1.117
ERROR			.183	.104	.175	.089	.209	.595	.203	.105	.089
LINURON	50	37	1.433	.301	.595	.522	-.256	.302	.769	.881	.833
LINURON	50	37	1.158	.325	.478	.492	-.421	.500	.797	.850	.907
LINURON	50	40	1.323	.414	.478	.991	-.033	.200	.725	.718	.803
LINURON	50	40	1.201	.404	.567	.880	-.236	.329	.834	.627	.903
MEAN			1.279	.361	.530	.721	-.236	.333	.781	.769	.861
ERROR			.124	.056	.061	.252	.159	.125	.046	.118	.052
LINURON	100	42	1.182	.338	.437	.371	-.538	-.671	-.324	.061	.096
LINURON	100	42	1.308	.214	.412	.454	-.738	-.727	-.239	.079	.036
LINURON	100	38	1.151	.571	1.270	1.314	-.478	-.620	.239	.692	.660
LINURON	100	38	1.149	.536	1.343	1.185	-.654	-.207	.368	.588	.710
MEAN			1.197	.415	.865	.831	-.602	-.556	.011	.355	.375
ERROR			.075	.169	.510	.487	.116	.237	.344	.332	.359
LINURON	500	39	1,455	.188	.823	.821	-.872	-.791	-.710	-.769	-.620
LINURON	500	39	1.303	.241	.705	.917	-.504	-.895	-.698	-.717	-.602
LINURON	500	33	1.255	.401	.614	1.087	-.847	-1.374	-.781	-.897	-.776
LINURON	500	33	1.229	.381	.647	1.192	-.941	-1.046	-.898	-.556	-2.518
MEAN			1.310	.303	.697	1.004	-.791	-1.027	-.772	-.735	-1.129
ERROR			.101	.104	.092	.167	.195	.254	.092	.141	.929
LINURON	1000	34	1.302	.009	.495	.535	-.870	-.939	-.894	-1.046	-1.004
LINURON	1000	34	1.107	.063	.506	.733	-.514	-1.152	-.977	-.960	-.962
LINURON	1000	35	1.226	.322	.772	.998	-1.081	-.922	-.914	-1.070	-1.064
LINURON	1000	35	.760	.443	.757	.957	-.675	-1.018	-.897	-1.029	-1.008
MEAN			1.099	.209	.632	.806	-.785	-1.008	-.920	-1,026	-1.009
ERROR			.240	.207	.153	.215	.245	.105	.039	.047	.042

CHAPTER IV

ATRAZINE UPTAKE, PHYTOTOXICITY, RELEASE AND SHORT-TERM RECOVERY FOR THE SUBMERGED AQUATIC PLANT, *POTAMOGETON PERFOLIATUS*

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ABSTRACT

Atrazine uptake, phytoxicity, release, and short-term photosynthetic recovery in the submerged vascular plant, Potamogeton perfoliatus, were investigated in laboratory studies. The processes of atrazine uptake and release were rapid, approaching equilibrium within 1 h. At low concentrations (<0.10 ppm), atrazine is accumulated into plant material at concentrations greater than those external to the plant. At the lowest concentration tested (0.01 ppm), the ratio of atrazine in the shoot to the incubation solution was 9:1. It is hypothesized that atrazine sorbs to plant tissue in addition to establishing an equilibrium between internal and external aqueous concentrations. Thus, at lower external atrazine solution concentrations, the sorbed herbicide exerts a greater influence on the ratio of shoot to solution atrazine causing the ratio to increase. The uptake of atrazine by roots of P. perfoliatus was found to be small in comparison to shoot uptake on a whole plant basis. The I_{50} (the concentration inhibiting photosynthesis by 50%) for atrazine in solution was determined to be 0.08 ppm with the maximum observed photosynthetic reduction (87%) at a solution concentration of 0.65 ppm. Initial photosynthetic recovery of P. perfoliatus following exposure to atrazine was rapid with oxygen evolution from treated plants (0.05, 0.03, and 0.10 ppm) being statistically indistinguishable ($p<0.05$) from control plants after 2 h of atrazine-free wash. However, there was an indication of residual photosynthetic depression in dosed plants, even after a 77 h recovery period. The ecological consequences of atrazine runoff into areas containing submerged aquatic vegetation must be evaluated in light of the ephemeral nature of the presence of atrazine in the water and the kinetics of uptake and release by the plants.

INTRODUCTION

A small but measurable percentage of the herbicides used for agricultural weed control is lost from the croplands where applied, and some eventually enters contiguous watercourses (Muir et al. 1978, Triplett et al. 1978, Wu 1980). If significant concentrations of these herbicides result in adjacent aquatic systems, a potential stress exists for the indigenous aquatic vegetation (Stevenson and Confer 1978). In many aquatic environments such as lakes and estuaries, a conspicuous and productive community is that of the submerged vascular plants. Over the last two decades, one such estuarine ecosystem, Chesapeake Bay, has experienced a marked decline in the abundance of this submerged aquatic vegetation (SAV). This decline occurred during a period of expanding use of herbicides, particularly atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) in the extensively cropped watershed (Bayley et al. 1978; Stevenson and Confer 1978). This coincidence of widespread herbicide use and SAV decline has been interpreted to suggest a possible causal connection between the two trends (Stevenson and Confer 1978; Correll et al. 1978).

Several recent studies have examined the effect of atrazine on SAV species in Chesapeake Bay (Forney and Davis 1981; Correll and Wu 1982; Cunningham et al. 1982). In each of these studies consistent significant losses of SAV growth and production were observed at atrazine concentrations of .05-.10 ppm. Concentrations of .01-.012 ppm resulted in significant effects on SAV in many (but not all) experiments. In nature, these submerged plants may be exposed ocassionally to atrazine concentrations on the order of .005-.050 ppm for brief periods during runoff events; however, dilution, adsorption, and degradation of the herbicide tend to reduce concentrations to 0-.005 ppm within 6-24 h (Correll et al. 1978; Kemp et al. 1982). Since SAV exposure to phytotoxic herbicide concentrations tends to be short term, it becomes important in assessing herbicide effects to know how rapidly the compounds are taken-up by plants, how uptake and plant response are related, and whether effects persist after herbicides are removed from the water.

The purpose of this study was to examine the kinetic relationships between SAV uptake of atrazine and phytotoxic response. Experiments conducted here involve the plant, *Potamogeton perfoliatus*, a dominant SAV species in upper Chesapeake Bay, and the herbicide, atrazine, which is the most widely used weed control chemical in the bay's watershed (Stevenson and Confer 1978). Specific questions investigated in this study include: 1) the rate of atrazine uptake; 2) the relative importance of root versus shoot uptake; 3) the relation between uptake and reduction in carbon assimilation; 4) the time required for photosynthetic recovery following brief (2 h) exposure to atrazine.

MATERIALS AND METHODS

Collection of Experimental Materials

Experimental plants (P. perfoliatus) were collected from shallow (0.5 to 1.5 m) areas of the Choptank River estuary, a tributary of Chesapeake Bay during July 1981 and 1982. Plants were obtained 1 d prior to experimental incubations by gently removing roots and rhizomes from their substrate and washing leaves and roots free of sediments and epiphytic material. Plants were held overnight in filtered estuarine water enriched with Provasoli's ES medium at 25 C under constant fluorescent illumination of 150 $\mu Ein \cdot m^{-2} \cdot s^{-1}$. All experiments were conducted either in a Percival environmental chamber or a microcosm system of 20 ℓ aquaria under the same light and temperature conditions, using filtered (0.45 μm) estuarine water of salinity similar to that at the site of collection (9-11 ppt).

Atrazine Uptake and Phytotoxicity

Matched sets of plant material (ca. 5 g wet wt) were selected for use in a series of 2 parallel treatments. The first treatment employed uniformly ring-labelled [^{14}C]-atrazine (50 μci/mg, CIBA-GEIGY Corp) dissolved in methanol to ascertain atrazine uptake, while the other used [^{14}C]sodium bicarbonate (53 μCi/mmol) plus non-labelled atrazine dissolved in methanol to determine the amount of CO_2-fixation at a given herbicide dosage. Plants were incubated in 2 ℓ Erlenmeyer flasks at atrazine concentrations of 0.01, 0.05, 0.10, 0.45, and 0.65 ppm. A methanol vehicle control containing [^{14}C] sodium bicarbonate plus the same amount of methanol as used with the atrazine and a standard control with [^{14}C] sodium bicarbonate only were also performed. The total volume in each flask was adjusted to 1 ℓ with ES medium, and all 4 flasks were simultaneously incubated for 4 h with periodic (hourly) mixing by hand swirling. At the end of the experiment plants were radioassayed as described below.

Time-Course Atrazine Uptake

Clean plants (terminal 20 cm) were placed in replicate flasks containing either 0.02, 0.05 or 0.10 ppm ^{14}C-atrazine (ring-labelled, 50 μci/mg) dissolved in 1.0 ℓ. The flasks were incubated for a total of 24 h at constant temperature and light. Plants were removed from the flasks at 0, 0.25, 0.50, 0.75, 1, 2, 4, 8, 12, and 24 h from the initial treatment, washed, and processed for radioassay as described below.

Photosynthetic Recovery

Experiments to test photosynthetic recovery were initiated by placing individually tagged plants in 300 ml BOD bottles containing filtered (1 μm) estuarine water. A control and 3 atrazine treatments (.005, .025, and .100 ppm) were used, with 5 replicate measurements of photosynthesis for each incubation. Bottles containing plants were suspended in 55 ℓ aquaria, with water circulated vigorously using submersible pumps. Apparent photosynthesis (P_a) was estimated by changes in dissolved oxygen (DO) during 2 h incubations, where DO measurements were made with a polarographic oxygen electrode (Orbisphere Model (2709).

Two sets of experiments were conducted to investigate photosynthetic recovery. In the first of these, dosed plants were transferred to atrazine-free filtered estuarine water in 300 ml BOD bottles at 2 h after initial atrazine dosing, and P_a was measured again in a second 2 h incubation. This was repeated two more times in new atrazine-free water, and then the tagged plants were returned to aquaria (one for each treatment) containing 20 ℓ of filtered water and maintained in the light overnight. A final 2 h incubation was conducted on the following day, 24 h after initial washing to estimate P_a. The second experiment involved washing plants in a larger volume of water and continued for more than 3 d. Plants were returned to the pre-incubation aquaria following the initial 2 h dosing period and allowed to flush in atrazine-free water (20 ℓ) for 3 h before being incubated in BOD bottles for 2 h to monitor photosynthesis. This procedure was repeated at 29 and 77 h. At the end of each time interval (3 h, 29 h, 77 h) the wash water in each aquarium was replaced with new filtered water.

Atrazine Release

Atrazine release was monitored for 0.10 ppm herbicide treated plants in the first of the recovery experiments above. Here, 10 replicate plants were treated for 2 h with 0.10 ppm ring-labelled ^{14}C-atrazine (50 μCi/mg). Five of these plants were immediately processed for radioassay as described below. The other 5 plants were placed in BOD bottles and treated as above. At the end of each incubation in atrazine-free water, 1 ml aliquots were taken from each BOD bottle and placed in 10 ml of Aquasol-2 (New England Nuclear) for subsequent radioassay. At the end of the entire series of incubations and washes, the plants were removed and dried for analysis of remaining ^{14}C content as described below.

The release of ^{14}C-atrazine was also measured in a wash series of various solvents. Plants incubated for 2 h in 0.01, 0.05, 0.10, 0.45 and 0.65 ppm ^{14}C-atrazine were subjected to a 4-step wash series. Four 1.0 ℓ beakers were arranged in the following order: (1) 500 ml of estuarine water, (2) 0.1 % Tween 80 (surfactant) in 500 ml of estuarine water, (3) 500 ml of estuarine water, and (4) 500 ml of a 2% HCl solution in estuarine water. The plants were sequentially placed and agitated briefly in each of the beakers, blotted dry, weighed, and dried at 80 C. Water from each of the 4 beakers was sampled (in triplicate) after each wash for ^{14}C-atrazine by placing a 1.0 ml aliquot into 10 ml Aquasol-2.

^{14}C-Analysis of Plant Material

All plant samples were dried at 80 C until constant weight (usually 24 h), separated into root/rhizome (referred to as roots) and stem/leaf (referred to as shoots) sections, weighed, and ground to powder with mortar and pestle. An aliquot (0.1-0.2 g) of the ground, dried plant material was placed in tubes and digested with nitric acid according to the method of Lewis et al. (1982). A portion (1.0 ml) of the digested plant material was placed in 10 ml of Aquasol-2. All counts were performed using a Packard Tri-Carb 460C Liquid Scintillation Spectrometer. Quench corrections were made using the external standard channels ratio method.

RESULTS AND DISCUSSION

Plant Uptake of Atrazine

The movement of atrazine into *P. perfoliatus* is quite rapid, coming to near equilibrium with the surrounding solution within 15 min (Fig. 1a). The atrazine concentrations within the plant remained quite stable from the first hour of incubation through the 24th hour (Fig. 1b). A one-way ANOVA with Student-Newman-Keuls (SNK) test of the means shows no significant difference ($p<0.05$) between plant atrazine concentrations past 15 minutes of incubation. Furthermore, varying solution concentrations of atrazine from .02 to .10 ppb had little or no effect in the time required to attain equilibrium.

There are few comparable studies involving herbicide uptake and subsequent release by aquatic plant species. The most similar studies are ones involving single-celled algae or bacteria. Vallentine and Bingham (1976) found that for several algae (*Scenedesmus*, *Chlamydomonas*, *Euglena*) the major uptake occurred within the first minute of incubation, with internal concentrations staying relatively constant for the remaining experimental period (24 h). Bohm and Muller (1976), also working with *Scenedesmus*, found that cellular atrazine uptake reached equilibrium with the culture solution in 4-8 h.

The relative importance of root versus shoot uptake of atrazine appears reasonably balanced (root uptake ranging from 16-40% of shoot uptake) when the data are normalized on a per gram tissue basis (Table 1). However, when the relatively low biomass of roots compared to shoots per plant is considered, uptake by the root portion of the plant is far less significant. In Table 1 uptake on a per gram root or shoot basis is compared to that on a total atrazine per plant part basis, and it is apparent that actual uptake of atrazine per plant by roots relative to shoots was very low. This estimate of root uptake is probably lower than that occurring in the natural environment due to possible injury to root systems during plant collection but still indicates a lesser role of the root system in uptake of atrazine. This is the same conclusion reached by Forney and Davis (1981) for both *P. perfoliatus* and *Elodea canadensis* where they partitioned roots and shoots, dosing one or the other or both.

Since the roots and shoots of the plants were suspended in the same concentration of atrazine and the shoots contained a relatively high concentration

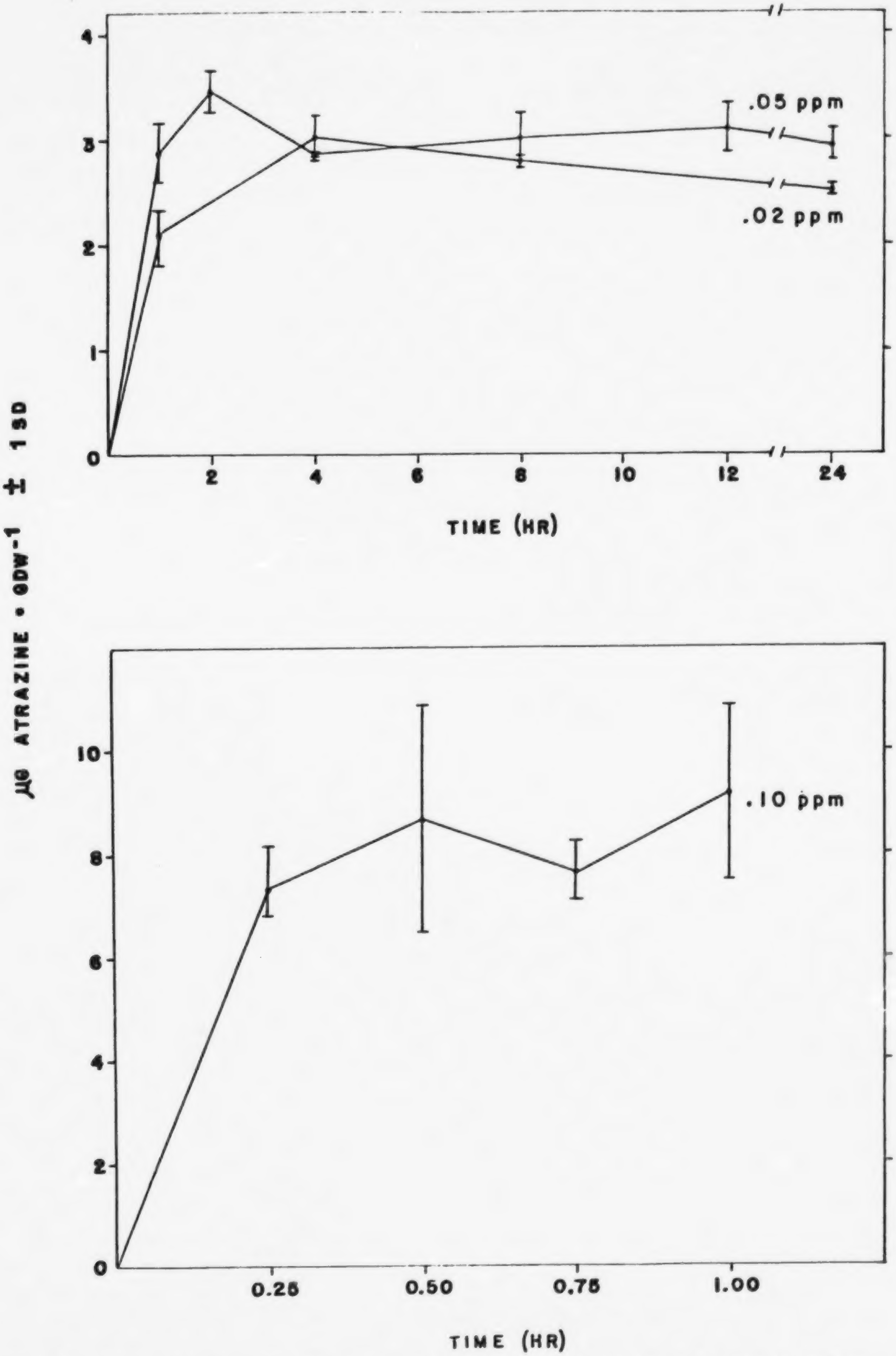

Fig. 1. Atrazine uptake by P. perfoliatus in (a) 24 hours at both 0.02 and and 0.05 ppm and (b) 1 hour at 0.10 ppm ($\bar{x}$ ± S.D.).

TABLE 1. Root:shoot ratio of ^{14}C-atrazine uptake in *P. perfoliatus.*

Atrazine Solution Concentration (ppm)	Root uptake per gram dry wt. / Shoot uptake per gram dry wt.	Root uptake per plant / Shoot uptake per plant
0.01	0.22	0.04
0.05	0.17	0.07
0.10	0.16	0.07
0.45	0.40	0.16
0.65	0.39	0.06

of atrazine, translocation of the compound from the roots to the shoots might have been inhibited. It is also not certain from these experiments whether there was any translocation at all. It is conceivable that some atrazine diffused initially from roots to shoots. However, the low final atrazine concentrations in the roots indicate that this was ultimately unimportant. Funderburk and Lawrence (1963) observed a movement of simazine from isolated roots to shoots in Heteranthera dubia (waterstargrass), but this was over a 5 d period compared to the 4 h incubation in the present study. Thomas and Seaman (1968) found no movement of simazine or atrazine when applied to the roots of Potamogeton nodosus and similarly no translocation of atrazine was observed from the roots of Potamogeton crispus (Sutton and Bingham 1968). Therefore, it might be concluded that translocation of significant quantities of atrazine by Potamogeton is doubtful.

Stem/leaf (shoot) uptake of atrazine (Fig. 2a) over the concentrations tested exhibited a generally linear pattern reaching 0.69 µg atrazine/gdw shoot (ppm) at 0.65 ppm aqueous concentration. Although plant dry weight is generally considered to be a more reliable estimate of plant biomass, wet weight was used in this study when considering atrazine uptake on a ppm basis. The wet weight to dry weight ratio of shoots in P. perfoliatus was determined to be 10.3 ± 1.2 (n = 25) and for roots was 8.1 ± 0.6 (n = 25). The water content, obviously a major portion of the biomass in a SAV species, must be considered when discussing diffusion of atrazine. Rhizome/root (root) uptake was also linear with the internal concentration reaching 0.27 ppm in a treatment of 0.65 ppm atrazine.

Atrazine Phytotoxicity

Inhibition of photosynthesis with increasing atrazine concentrations follows saturation kinetics with the maximum inhibitions (87 and 88%) occurring at 0.45 and 0.65 ppm, respectively (Fig. 2a). Dark CO_2-fixation for these plants was determined to be 2% of the total CO_2 fixed in the light, and uptake of atrazine was the same in the light as in the dark (this uptake insensitivity to light for atrazine uptake has also been reported by Vallentine and Bingham (1976) working with the alga Scenedesmus). The I_{50} (atrazine concentration at which photosynthesis is inhibited 50%) for P. perfoliatus in these experiments was 0.08 ppm. This is in relatively good agreement to the I_{50} found by Cunningham et al. (1982) who worked with plants from the same P. perfoliatus population as in this study. They reported a 50% reduction in apparent photosynthesis (P_a) at an atrazine concentration of 0.13 ppm. Of interest is the fact that their experiments were long term (3 wks) and used oxygen evolution to assess photosynthesis as compared to the experimental time (4 h) and methodology (^{14}C) of our study.

Outside of the work by Cunningham et al. (1982), it is difficult to compare the results of this study on P. perfoliatus with others. Forney and Davis (1981) did use as one of their SAV plants P. perfoliatus but measured changes in plant length, dry weight and mortality as indices of atrazine toxicity. They estimated the I_{50} in one experiment to be 0.474 ppm for length and 0.091 ppm for dry weight; however, in another experiment of longer duration (4 wk) a 51% reduction in elongation rate occurred at 0.032 ppm. With Elodea canadensis, values of I_{50} were obtained at 0.075 ppm and 0.123 ppm for elonga-

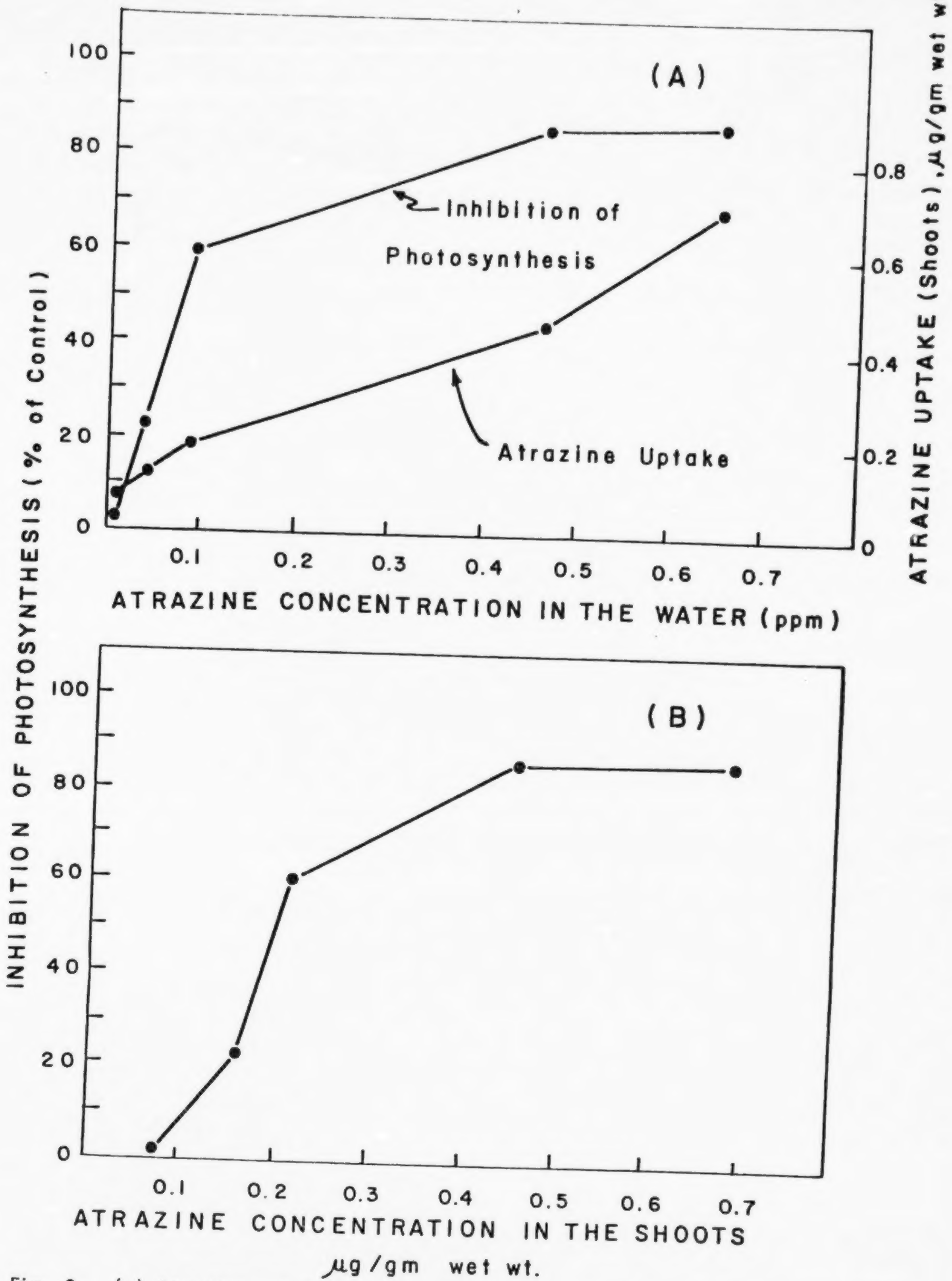

Fig. 2. (a) Atrazine uptake and associated photosynthetic inhibition in P. perfoliatus at atrazine concentrations of 0.01 to 0.65 ppm. (b) Internal atrazine concentration and associated photosynthetic inhibition in P. perfoliatus.

tion and weight increment, respectively. While Correll and Wu (1982) observed a slight stimulation of apparent photosynthesis for P. pectinatus at 0.075 ppm atrazine, reductions of 50% and 20% were found for Zannichellia palustrus and Vallisneria americana, respectively, at that concentration. Thus, it would appear that atrazine concentrations on the order of 0.100 ppm would effect an I_{50} for most SAV species studied.

The relationship of internal atrazine concentration in the shoot to photosynthetic inhibition shows that the maximum photosynthetic inhibition observed occurs when the internal atrazine concentration of the shoots reaches about 0.45 ppm (Fig. 2b). A striking relationship is evident between the external and internal atrazine concentrations in the shoots and the degree of photosynthetic inhibition. An equilibrium appears to exist between internal and external atrazine concentration only at the point in which maximum photosynthetic inhibition was observed. When the other atrazine solution concentrations tested are examined, a slightly different case is found. In Fig. 3 it can be seen that the ratio of atrazine in the shoot to atrazine in solution is near unity at 0.45 ppm and increases (shoot accumulation) with lower concentrations. If atrazine uptake in plants is a passive diffusion process, one would expect (barring significant degradation in a 4 h period) an equilibrium to be established between the total internal and external ^{14}C-activity producing a ratio of unity. However, the ratio for the shoots is always greater than one indicating a possible atrazine accumulation against a concentration gradient. This relative acccumulation increased as the solution atrazine concentration decreased. The plant:solution ratios for roots versus atrazine concentrations (Fig. 2) are all less than unity (with the exception of the roots in the 0.01 ppm solutions). Thus reflecting the relatively poor absorption of atrazine by the P. perfoliatus roots in these experiments.

This apparent accumulation of atrazine at low concentrations has been observed in algae by direct measurement (Bohm and Muller 1976) and indirectly by measurement of the % atrazine remaining in solution (Vallentine and Bingham 1976). Atrazine is known to have a high binding affinity for organic matter (Weber et al. 1969). The apparent accumulation at low atrazine concentrations seen in this study and those of Bohm and Muller (1976) and Vallentine and Bingham (1976) could simply be a result of the fixed amount of atrazine sorbed per gram dry weight plant material having a greater influence on the ratio of internal to external atrazine as the external atrazine concentration decreases. In support of this is the report by Geller (1979) who found a good correlation between bacterial surface area and atrazine accumulation, i.e., increased surface area yielded higher atrazine accumulation.

Another possible explanation for this apparent accumulation of atrazine in the shoots of P. perfoliatus at low atrazine concentrations may be found in the fact that photosynthetic inhibitors like atrazine bind to protein at their sites of activity in the chloroplast (Pfister et al., 1980). The total number of binding sites for atrazine in the chloroplasts of the plant would be a finite number relative to the mass of photosynthetic units in the plant (the number of sites is estimated to be between several hundred to twenty-five hundred per chlorophyll molecule (Izawa and Good, 1965; Tischer and Strotmann et al. 1974)). As atrazine diffuses into the plant chloroplasts and is bound to the sites it would be removed from the solution phase thus reducing the

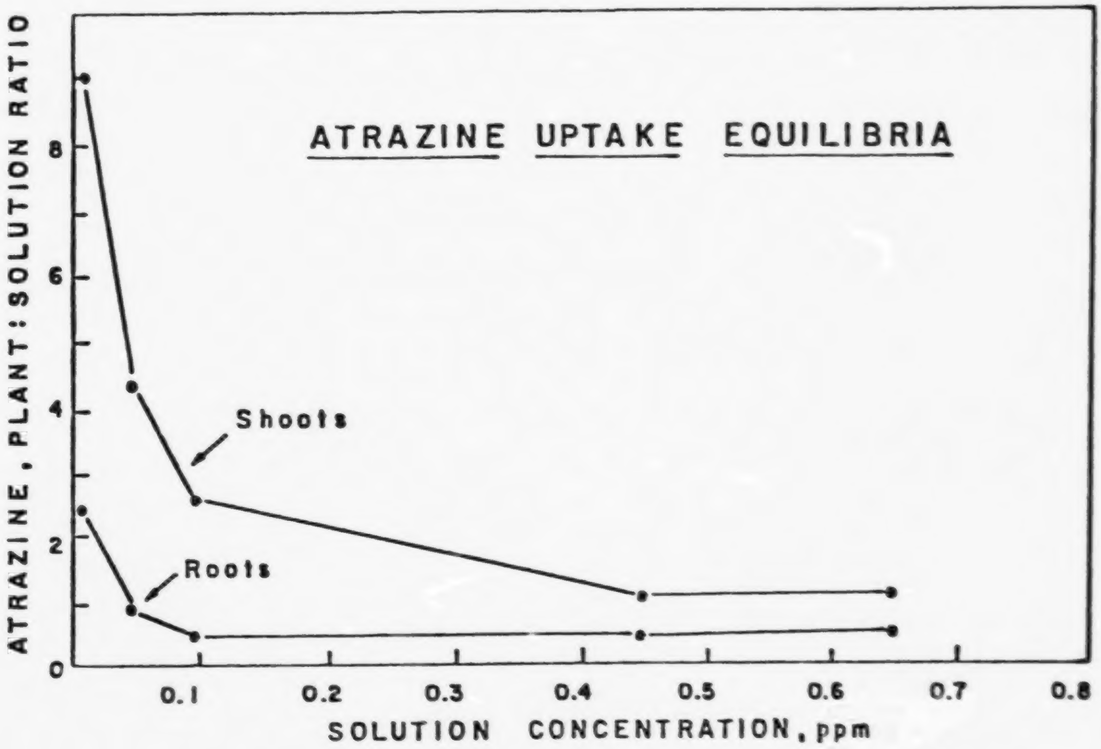

Fig. 3. Ratio of atrazine uptake in *P. perfoliatus* to atrazine concentration in the medium.

plants incubated for 4 hr in a concentration gradient of atrazine is shown in Table 2. The purpose of using this wash sequence was to see the degree of sorption of ^{14}C-atrazine in the plant material. It was assumed that first wash would remove the loosely associated surface film containing the ^{14}C-atrazine from the incubation solution, while the surfactant in the second wash would remove physically bound chemicals. The third wash (filtered water only) was simply to remove the surfactant from the plant and as a check on the amount of ^{14}C-atrazine associated with the plant still removable by a water wash alone. This would provide a somewhat clearer picture of the absolute effect of the 4th wash, the 2% HCl solution which was intended to shock the membrane electrical potential allowing materials in the cytoplasm to flow out.

The first water wash removed the most ^{14}C-atrazine. The surfactant wash removed very little additonal herbicide and may just represent a carry-over phenomenon from the first wash water. However, the acid wash did remove a substantial amount of ^{14}C-atrazine. The percentages in parentheses in Table 2 after the atrazine quantities indicate herbicide removal relative to the total internal concentration (no percentages are given for the first wash due to the variability of the amount of carry-over water from the incubation solutions). The low percentage removed by wash #3 (just water) over the range of atrazine concentrations tested indicates the thoroughness of the previous washes in removing externally sorbed atrazine.

The exact physio-chemical mechanisms of atrazine removal brought about by the acid wash and the location of the atrazine within the plant material are open questions. One explanation is that atrazine is hydrolyzed to hydroxyatrazine by acid pH, facilitating the dissolution of this compound, which is relatively more polar than atrazine. Therefore, the observed release of ^{14}C-atrazine (ring-labelled) could have been an observation of ^{14}C-hydroxyatrazine going into solution only. Nevertheless, the high percentage of ^{14}C-atrazine removed by the acid wash (37%) does indicate that a substantial quantity of atrazine sorbed by the plant material is located superficially.

Photosynthetic Recovery

The photosynthetic recovery of *P. perfoliatus* occurs as rapidly as uptake and release of atrazine. In both experimental procedures, the plants recovered to greater than 75% of their maximum photosynthetic potential within the measured time of 2-3 h (Fig. 4a,b). A rapid rate of recovery was apparent within the first 2 h, with a slower recovery occurring over the next 10-80 h. Although a SNK test shows no significant difference ($p<.05$) between the means after 2-3 h, a slow but steady increase in photosynthesis is evident (the reason for the drop in photosynthesis seen in the 0.025 ppm treatment (Fig. 4a) is unknown. However, as seen in Fig. 4a, photosynthesis is still depressed in the low volume wash experiment after 24 hours (a significant difference at $p<0.25$ at all concentrations). Even after 77 h of flushing in the large volume experiment photosynthesis is still less than control plants in the .10 ppm treated plants (Fig. 4b). However, analysis of the plants in the first recovery procedure exposed to 0.10 ppm of ^{14}C-atrazine indicated that they contained no significant quantity of ^{14}C at the end of 24 h of flushing.

Cunningham et al. (1982) observed a partial recovery of photosynthesis by

TABLE 2. The quantity of ^{14}C-atrazine (μg) removed from *P. perfoliatus* by the various washes following the four-hour incubation period.

Atrazine Solution Concentration	#1 Water	#2 Surfactant	#2 Water	#4 2% HCl
0.01	0.14	0.01 (3)[a]	0.00 (0)	0.02 (5)
0.05	0.10	0.03 (5)	0.00 (0)	0.18 (29)
0.10	0.26	0.09 (11)	0.00 (8)	0.29 (37)
0.45	1.14	0.38 (16)	0.19 (8)	0.45 (19)
0.65	1.54	0.41 (13)	0.20 (8)	0.60 (19)

[a] Numbers in parentheses are the percent of total plant ^{14}C-atrazine removed.

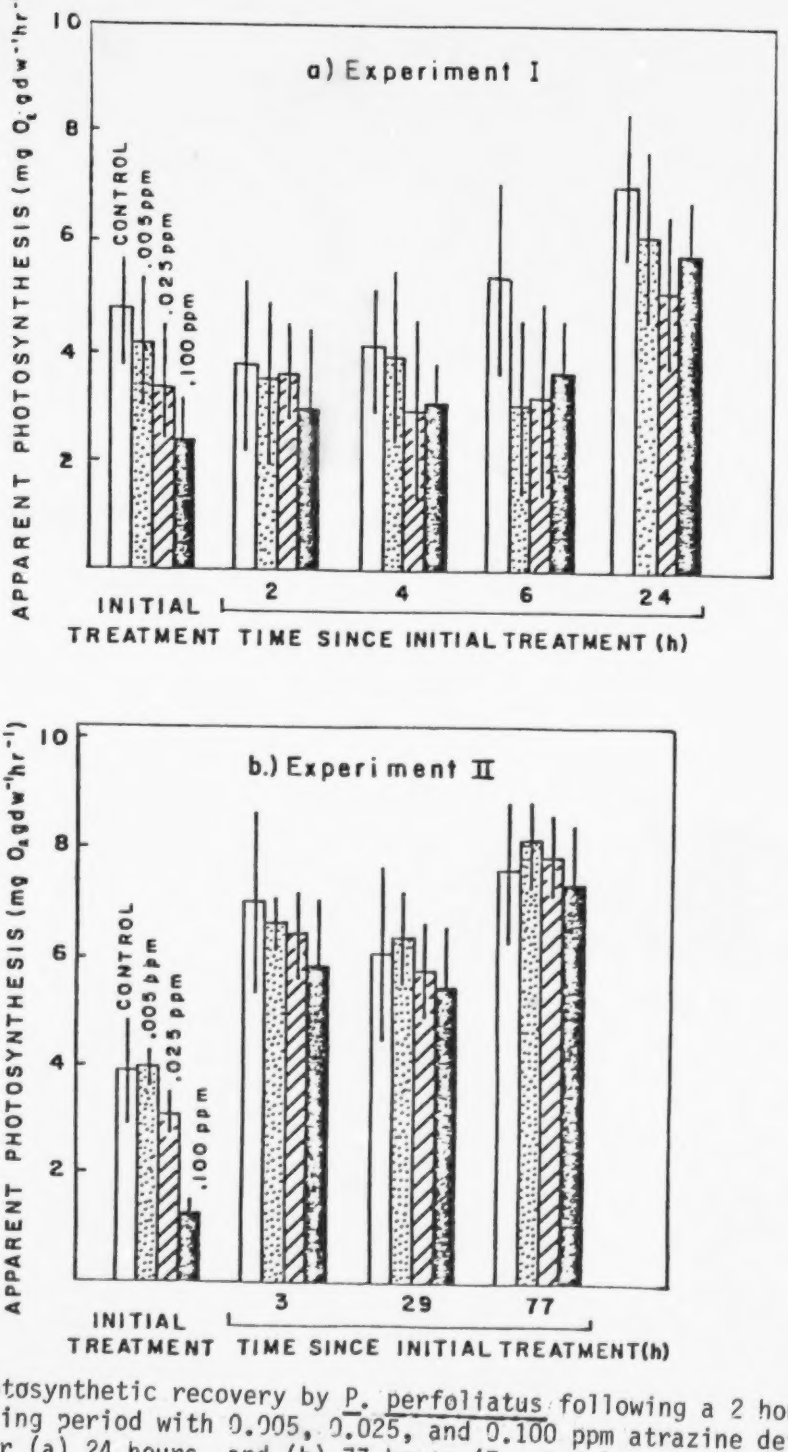

Fig. 4. Photosynthetic recovery by *P. perfoliatus* following a 2 hour dosing period with 0.005, 0.025, and 0.100 ppm atrazine determined over (a) 24 hours, and (b) 77 hours ($\bar{x}$ ± S.D.).

P. perfoliatus after the initial atrazine dosing in their microcosm study. In their study, however, the recovery (P_a as measured by oxygen changes in the microcosms) occurred 1 wk after dosing (0.13 ppm) while atrazine was still present in the microcosm water (the atrazine concentration declined only 15% during the 4 wk post-treatment period). Although the experimental conditions of their system and those of the present study were quite different, the combined results do indicate the resilience of P. perfoliatus to atrazine exposure.

CONCLUSIONS

The results obtained in this study indicate that Potamogeton perfoliatus is a relatively sensitive plant to atrazine (I_{50} = 0.075 ppm) when grown at 10 ppt salinity. Root uptake of atrazine was small in relation to shoot uptake (~6 percent of shoot), and therefore, when considering short term photosynthetic depression in SAV species, it is the atrazine in solution around the plant shoots which is of most concern. Uptake and release of atrazine is rapid (ca. 1 h), but there is an indication of some residual photosynthetic inhibition as long as 77 h following dosing eventhough no detectable atrazine could be found in the plants.

The sorption of atrazine to SAV surfaces (as well as algal surfaces) leads to amounts of atrazine associated with aquatic plant materials in higher concentrations than that observed in the adjacent waters. This conceivably could serve as a mechanism for bioaccumulation of atrazine in aquatic food webs. However, Davis et al. (1979) in a study of the fate of atrazine in a salt marsh ecosystem found no accumulation of atrazine in the animals (e.g., crabs, snails) allowed to graze the dosed systems.

In regard to the role of atrazine in the decline of SAV in the Chesapeake Bay, it would appear from these data and the observed concentrations of atrazine in the Bay that photosynthetic losses would be ephemeral allowing the plants to recover. The rapid degradation of atrazine in estuarine environments (Jones et al. 1982) along with sorption to sediment and dilution through tidal actions would keep SAV species from being exposed to lethal concentrations of the herbicide. However, the ultimate effects of the presence of atrazine in the water and the associated short-term photosynthetic losses on the biology of the plants is unknown. Especially of concern is any effect on reproductive success.

REFERENCES

1. Bayley, S., Stotts, V.D., Springer, P.F., and J. Steenis. 1978. Changes in submerged aquatic macrophyte populations at the head of the Chesapeake Bay, 1958-1975. Estuaries 1:74-75.

2. Bohn, H.H. and H. Muller. 1976. Model studies on the accumulation of herbicides by microalgae. Naturiwissenschaften 63:296.

3. Correll, D.L., Pierce, J.W., and T.L. Wu. 1978. Herbicides and submerged plants in the Chesapeake Bay. Pages 858-877 *in* Proc. Symp. on Technicol., Environmental, Socio-economical, and Regulatory Aspects of Coastal Zone Management. Am. Soc. Cw. Eng.

4. Correll, D.L. and T.L. Wu. 1982. Atrazine toxicity to submersed vascular plants in simulated estuarine microcosms. Aquatic Bot. (In press).

5. Cunningham, J.J., Kemp, W.M., Stevenson, J.C., and M.R. Lewis. 1982. Patterns of ecological and physiological response and recovery of the submerged macrophyte, *Potamogeton perfoiliatus* exposed to atrazine stress in estuarine microcosms. Report to U.S. EPA, Annapolis, MD.

6. Davis, D.E., Weete, J.D., Pillai, C.G.P., Plumley, F.G., McEnerney, J.T., Everest, J.W., Truelove, B., and A.M. Diner. 1979. Atrazine fate and effecgts in a salt marsh. EPA report no. 600/3-79-111. 84 pp.

7. Forney, D.R., and D.E. Daves. 1981. Effectgs of low concentrations of herbicides on submersed aquatic plants. Weed Sci. 29:677-85.

8. Funderburk, H.H. Jr., and J.M. Lawrence. 1963. Absorption and translocation of radioactive herbicides in submersed and emersed aquatic weeds. Weed Res., 3:304-311.

9. Geller, A. 1979. Sorption and desorption of atrazine by three bactgerial species isolated from aquatic systems. Arch. Environ. Contam. Toxicol. 8:713-720.

10. Izawa, S. and N.E. Good. 1965. The number of sites sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethyurea,3-(4-chlorophenyl)-1,1-dimethylurea and 2-chloro-4-(2-propylamino)-6-ethylamino-*s*-triazine in isolated chloroplasts. Biochim. Biophys. Acta 102:20-38.

11. Jones, T.W., Kemp, W.M., Stevenson, J.C. and J.C. Means. 1982. Degradation of atrazine in estuarine water/sediment systems and soils. J. Environ. Qual. 11:632-638.

12. Kemp, W.M., Means, J.C., Jones, T.W., and J.C. Stevenson. 1982. Herbicides in Chesapeake Bay and their effects on submerged aquatic vegetation. Univ. Maryland Centr. Environ. Est. Stud. Ref. No. 1295-HPEL. Horn Pt. Env. Labs., Cambridge, Md. 82 pp.

13. Lewis, M.R., Kemp, W.M., Cunningham, J.J., and J.C. Stevenson. 1982. A rapid technique for preparation of aquatic macrophyte samples for measuring ^{14}C incorporation. Aquatic Bot. 13:203-207.

14. Muir, D.C.G., Yoo, J.Y., and B.E. Baker. 1978. Residues of atrazine and N-de-ethylated atrazine in water from five agricultural watersheds in Quebec. Arch. Environ. Contam. Toxicol. 7:221-224.

15. Pfister, K., Steinback, K.E., Gardner, G., and C.J. Arntzen. 1980. Identification of the photosystem II Herbicide binding protein. Pl. Physiol 65(6): Supplement, Abstr. No. 48.

16. Stevenson, J.C. and N.M. Confer. 1978. Summary of available information on Chesapeake Bay submerged vegetation. U.S. Department of Interior, Fish and Wildlife Service. (FWS/OBS-78/66). Washington, D.C. 335 pp.

17. Sutton, D.L. and S.W. Bingham. 1968. Translocation patterns of simazine in *Potamogeton crispus* L. Proceed. N.E. Weed Control Conf. 22:357-361.

18. Tischer, W. and H. Strotmann. 1977. Relationship between inhibitor binding by chloroplasts and inhibition of photosynthetic electron transport. Biochem. Biophys. Acta 460:113-125.

19. Thomas, T.M. and D.E. Seaman. 1968. Translocation studies with Endothal-^{14}C in *Potamogeton nodosus* Poir. Weed Res. 8:321-326.

20. Triplett, G.B., Conner, B.J., and W.M. Edwards. 1978. Transport of atrazine and simazine in runoff from conventional and no-tillage crops. J. Environ. Qual. 7:77-84.

21. Vallentine, J.P. and S.W. Bingham. 1976. Influence of algae on amitrole and atrazine residues in water. J. Can. Bot. 54:2100-2107.

22. Weber, J.B., S.B. Weed, and T.M. Ward. 1969. Adsorption of *s*-triazines by soil organic matter. Weed Sci. 17:417-421.

23. Wu, T.L. 1980. Dissipation of the herbicides atrazine and alachlor in a Maryland corn field. J. Environ. Qual. 9:459-465.

CHAPTER V

UPTAKE AND PHYTOTOXICITY OF SOIL-SORBED ATRAZINE FOR THE SUBMERGED AQUATIC PLANT, *POTAMOGETON PERFOLIATUS*.

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ABSTRACT

The effect of atrazine-sorbed soil on photosynthesis in *Potamogeton perfoliatus* was investigated under laboratory conditions. Leaves simultaneously exposed to atrazine both in solution and sorbed to soil sedimented on the leaves exhibited a similar uptake of atrazine and associated photosynthetic reduction as did leaves exposed to the same concentration of atrazine in solution only. A small quantity of atrazine (0.19 µg/gdw leaf) was found in leaves treated with atrazine-sorbed soil at 120 ppb whereas a significantly larger amount (3.57 µg/gdw leaf) was present in leaves treated with dissolved atrazine at a concentration of 100 ppb. Based on these results, it is concluded that atrazine sorbed to soil is less available for uptake by aquatic plants than atrazine in solution. Of greater physiological concern is the physical presence of the soil on the leaves and the resultant attenuation of light.

INTRODUCTION

The ecological effects of herbicide runoff into estuarine aquatic environments like the Chesapeake Bay have received considerable attention recently with a special emphasis toward the impact on submerged aquatic vegetation (SAV) (Stevenson and Confer 1978, Correll et al. 1978). Triazine herbicides such as atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) are relatively mobile in the soil (Wauchope and Leonard, 1980) and do move from agricultural fields. It has been observed that approximately 1% of the atrazine applied to a field may ultimately enter nearby aquatic systems (Muir et al., 1978; Triplett et al., 1978; Wu, 1980), most of which occurs during the first major storm event following application (Muir et al., 1978; Triplett et al., 1978; Wauchope, 1978). This runoff can result in dissolved atrazine concentrations ranging from less than 1 to 100 ppb in adjacent waters (Frank et al., 1979; Frank and Sirons, 1979; Wu, 1980; Hershner et al. 1981).

The partitioning of atrazine between the dissolved phase and the soil-sorbed phase in the run-off component may be of key importance as to the availability of the herbicide for uptake by SAV species. Although the partition coefficient (Kd = sorbed concentration ÷ dissolved concentration at equilibrium) for atrazine is variable due to soil parameters (most notably organic matter, pH, and clay content), average values for many agricultural soil types are usually between 1 - 5 (Talbert and Fletchall, 1965). Partition coefficients of 1 - 4 have also been reported for estuarine sediments (Means et al., 1980). However, substantially higher Kd values (5-260) for suspended sediment in run-off have been calculated (Correll and Wu, 1982). Since the Kd values are routinely greater than 1, there is the potential for concentration of atrazine within the suspended particulate fraction of field run-off material. The accumulation of this atrazine-sorbed sediment produces a microenvironment on SAV leaf surfaces that theoretically could result in a high concentration of atrazine in the interstitial water of that sediment. Therefore, it has been suggested that atrazine sorbed to suspended particulates may, in effect, result in SAV exposure to elevated herbicide concentrations by sedimentation of this material onto leaf surfaces (Correll and Wu, 1982).

This research was initiated to examine the extent of uptake of atrazine in *P. perfoliatus* when the soil-sorbed herbicide is placed on plant leaves. Atrazine uptake and the resulting photosynthetic depression in *P. perfoliatus* leaves were investigated under the following conditions: 1) atrazine in solution and on the overlying soil, 2) atrazine in solution only, 3) atrazine on the overlying soil only.

MATERIALS AND METHODS

Sorption - Desorption of Atrazine on Soil

The soil used throughout this study was Mattapex silt-clay collected from an agricultural field that had not been tilled for four years. Two particle size classes were tested for their sorptive properties. One class contained particles which passed through a 105 μ sieve, but not through a 74 μm sieve. The other contained particles which would pass through a 74μm, but not a 53μm sieve. These sizes were chosen because they could be suspended in a column of water and yet would settle out within five minutes, suggesting that when suspended in an aquatic environment, these particles might easily settle on the leaves of submerged macrophytes.

Sorption of atrazine was examined at a water:soil ratio of 5:1 using five concentrations of uniformly ring-labelled ^{14}C-atrazine in methanol (10, 50, 100, 500, 1000 ppb). Two-gram soil samples were added to 16 x 150 mm glass screw cap tubes to which 10 ml of atrazine solution was then added. The soil samples were allowed to equilibrate for 6 hours on a mechanical shaker at room temperature after which they were centrifuged. One-ml supernatant water samples were taken from each tube and placed into 10 ml of Aquasol-2 (New England Nuclear) for counting on a Packard Tri-Carb Model 460C Liquid Scintillation spectrometer. The difference between the amount of atrazine originally in solution and the amount remaining in solution after incubation was assumed to be the amount sorbed to the soil.

After water samples were removed from the tubes, the remaining supernatant was aspirated off and replaced with 10 ml of deionized water. The tubes were shaken for 2 hours, centrifuged, and sampled as before to determine the amount of atrazine which had desorbed from the soil. This entire procedure was repeated to determine second-degree desorption. Wet weights and then dry weights of the soil samples were taken to determine the interstitial water volume which was used to correct desorption values.

Plant Preparation

Potamogeton perfoliatus (L) plants were collected from shallow waters of the Choptank River estuary just prior to each experiment. Epiphytes and sediments were removed from the leaves manually and selected leaves from the terminal 20 cm of at least 10 different plants were removed from the stems and placed in filtered (.45 μm) Choptank water.

Leaves were arranged in four rows on a rack consisting of a ring of PVC pipe (150 mm inside diameter, 43 mm high) with a black plastic mesh (2.75 mm) bottom with three supporting legs (15 mm high). Four strands of monofilament

In Treatment #2 leaves were exposed to 120 ppb atrazine on the soil with no atrazine in solution. The other two bowls were identical to those in Treatment #1. In Treatment #3, soil was applied without sorbed atrazine (methanol served as a vehicle control), however, the water did contain 100 ppb atrazine. Again the other bowls were identical to Treatments #1 and #2.

RESULTS & DISCUSSION

The two particle size classes of Mattapex silt-clay examined for use in this study exhibited similar adsorptive (Fig. 1) and desorptive (Fig. 2) characteristics. Ultimately, the larger size class (<105μm, >74μm) was chosen over the smaller (<74μm, >53μm) because the former has a greater tendency to remain settled on P. perfoliatus leaves during the experimental procedures. This larger size class has a partition coefficient (K_d) for atrazine of 1.2 ($r^2 = .99$).

The importance of achieving a uniform distribution of soil over the leaves of a plant and the necessity of being able to apply and recover known quantities of soil was addressed by the use of detached leaf experiments. Atrazine uptake rates (in dissolved phase) by attached and detached leaves of P. perfoliatus were not significantly ($p<0.05$) different. The mean uptake of atrazine at a dissolved concentration of 120 ppb (μg atrazine/gdw leaf) for attached leaves was 6.31 ± 1.32 ($\bar{x}$ ± S.D.) and 5.97 ± 1.51 for detached leaves. However, attached leaves did exhibit a higher mean photosynthetic rate (10.74 ± 2.01 mgC/gdw/hr) than did detached leaves (6.94 ± 1.93 mgC/gdw/hr). Since only relative photosynthetic differences among treatments was considered to be of importance here, this photosynthetic rate difference was of no consequence.

The results of experiments on atrazine uptake by leaves of P. perfoliatus from sorbed-soil versus aqueous solution indicate the relative low availability of soil-sorbed atrazine for plant uptake (Fig. 3). In Treatment 1, leaves that were exposed to atrazine-sorbed soil and dissolved atrazine simultaneously (at the proper $K_d = 1.2$) exhibited no significant difference ($p<.01$) in uptake from leaves exposed to dissolved atrazine only (3.32 ± 0.31 versus 3.58 ± 0.21 μg atrazine/gdw leaf, respectively). When leaves were exposed to atrazine-sorbed soil only (Treatment 2), uptake was minimal (0.19 ± .03 μg/gdw) as compared with uptake by leaves without soil exposed to an equal concentration of dissolved atrazine (3.57 ± .11 μg/gdw). The physical presence of soil overlying the leaves did not impede or promote the uptake of atrazine from solution (Treatment 3). The uptake by leaves with applied soil (no atrazine) and exposed to dissolved atrazine alone did not significantly differ ($P<.01$) from the atrazine uptake by leaves without soil exposed to issolved atrazine (4.55 ± .013 and 4.31 ± .06 μg atrazine/gdw, respectively).

The results obtained in the atrazine-sorbed soil treatments above are consistant with reported atrazine adsorption-desorption kinetics in relation to water:sediment ratios. As this ratio decreases, atrazine sorption to the same sediment (same K_d) increases (Laskowski et al. 1980). The water:sediment ratio in the microenvironment of pore-waters between adjacent sediment particles accumulated on SAV leaf surfaces would be extremely low causing the atrazine

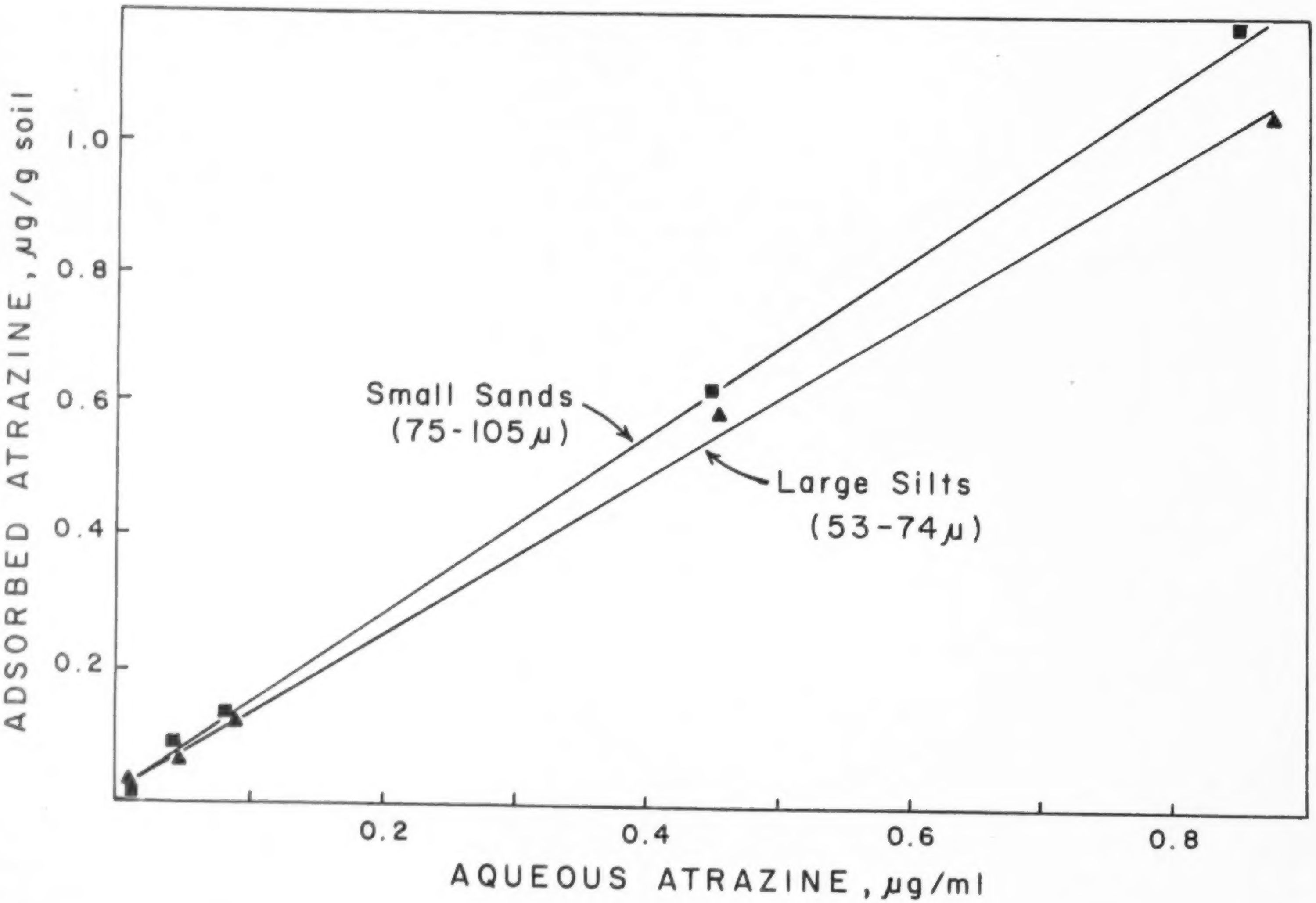

Figure 1. Atrazine adsorption on 2 particle sizes (53-74 μ, 75-105 μ) of Mattapex silt-clay at a water:soil of 5:1.

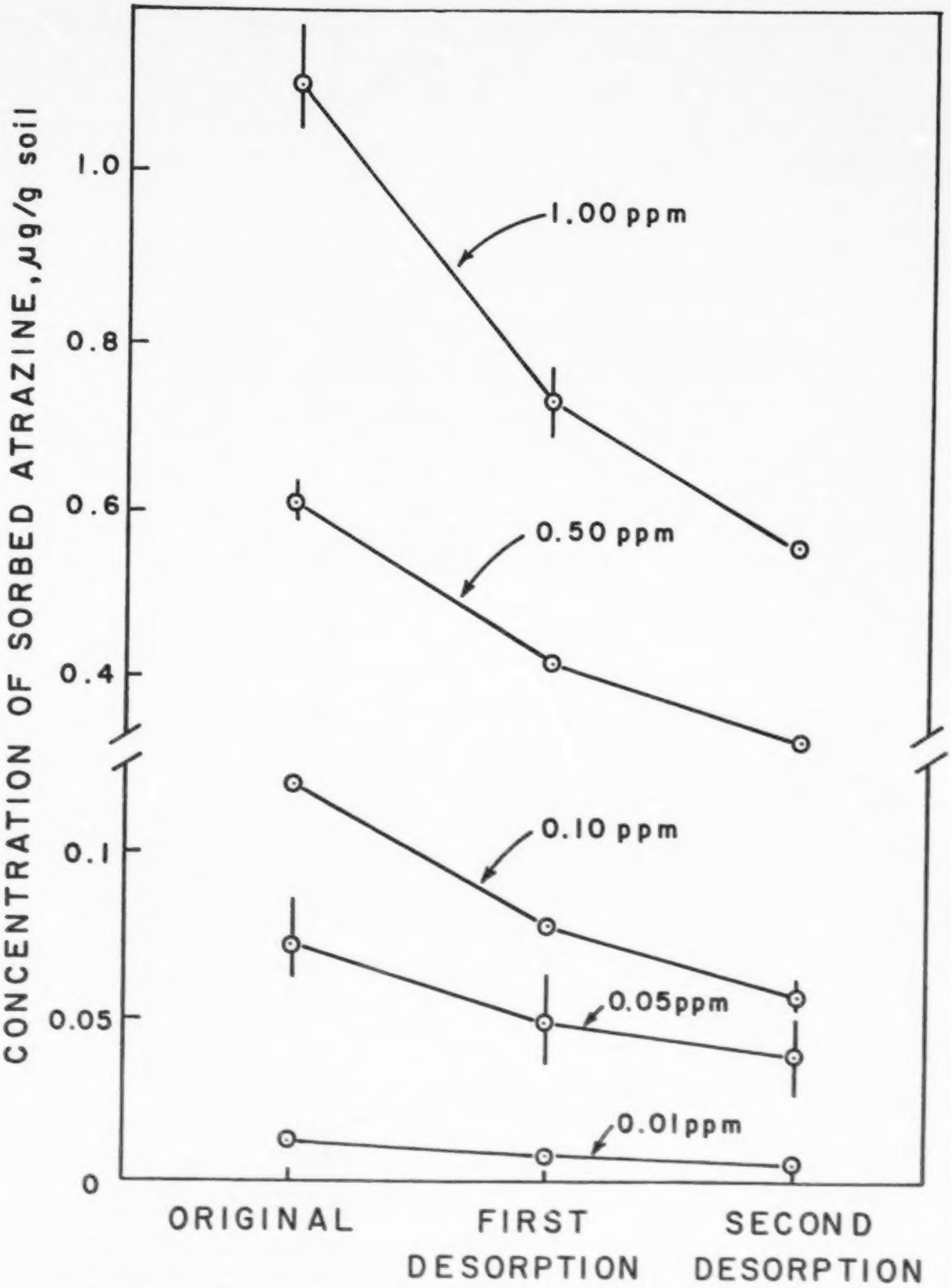

Figure 2. Atrazine desorption from Mattapex silt-clay at a water:soil of 5:1. Error bars represent range between the particle sizes uses (53-74 μ and 75-105 μ).

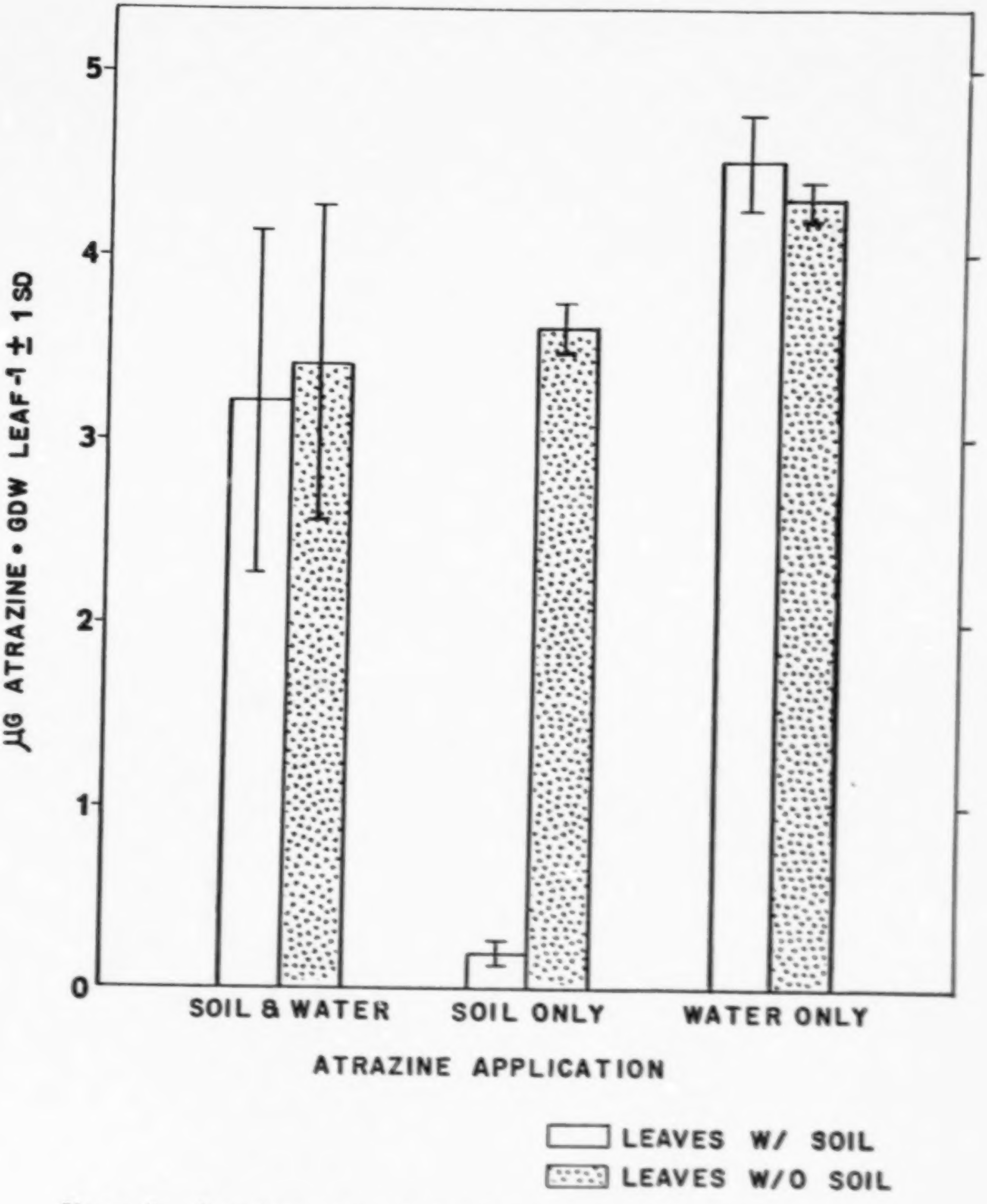

Figure 3. Atrazine uptake in leaves of P. perfoliatus from atrazine-sorbed soil and from solution ($\bar{x}$ ± S.D.).

to remain sorbed to the sediment. However, the upper surfaces of the accumulated sediment would be exposed to the larger volume of water in the system and the atrazine would desorb accordingly.

The photosynthetic response of P. perfoliatus leaves to shading by varied amounts of applied soil (0 to 9.93 g soil/gdw leaf) was quite variable (Fig. 4) and non-linear, appearing not to increase past about 4 g soil/gdw leaf at which point the mean photosynthetic reduction was 28%. When atrazine and surface soil are present in a system, the resulting photosynthetic reduction is due to both physical shading of the leaves by the soil and to the presence of atrazine (dissolved and/or sorbed to the soil). The mean value of 28% photosynthetic reduction brought about by surface soil in the range of 4-10 g soil/gdw leaf Fig. 4) was subtracted from the total photosynthetic reduction derived experimentally to obtain the reduction due to atrazine alone (Table 1). When atrazine was present in solution, the resulting photosynthetic reduction ranged from 52 to 69%. Additional atrazine sorbed to the surface soil did not seem to result in any additive photosynthetic reduction. Also, when atrazine was introduced on the soil only, very little photosynthetic reduction resulted, again indicating that atrazine-sorbed soil was not taken up by the leaves of P. perfoliatus.

It appears that the most important effect of soils (with or without atrazine) on plant leaves is attributable to the physical presence of sediment on leaf upper surfaces. Published data on the effects of sediment on SAV leaf surfaces on photosynthesis is wanting. However, data on epiphyte biomass on SAV leaves are available. Epiphytes exert a similar influence on SAV species as does settled soil in that they attenuate light. It has been shown that light to the leaf surface of Zostera maximum can be reduced by 90% by natural epiphytic growth (Phillips et al. 1978, Borum and Wium-Andersen 1980) and photosynthesis in Zostera can be reduced by as much as 31% (Sand-Jensen 1977) by overlying epiphytes. Twilley et al. (1982) found a 27% reduction in SAV photosynthesis with epiphyte communities of 6.13 grams dry wt epiphyte per gram dry wt SAV. These values are surprisingly close to the maximum amounts of soil (4-9 grams per gram dry wt leaf) applied to the leaves of the present study and the average SAV photosynthetic reduction calculated (27%, Table 1). Apparently densities of naturally occurring epiphytes of the same weight as soil on leaf surfaces correlate well in relation to photosynthetic reduction.

In conclusion, it would appear from the data of this study that soil-sorbed atrazine is relatively unavailable for uptake by P. perfoliatus. Desorption of atrazine from soil is rapid, and therefore, it is likely that the soil deposited on SAV leaves would have an atrazine concentration in equilibrium (K_d) with the water. Forney and Davis (1981) came to a similar conclusion following their study of 4 Chesapeake Bay SAV species and atrazine. They too found that atrazine uptake from the water was the main mode of entry of the herbicide into the plant. Furthermore, atrazine degrades more rapidly to hydroxyatrazine (nonphytotoxic) when it is in close proximity to soil surfaces (Armstrong and Chesters 1968) so that the soil on leaf surfaces could even serve to detoxify atrazine.

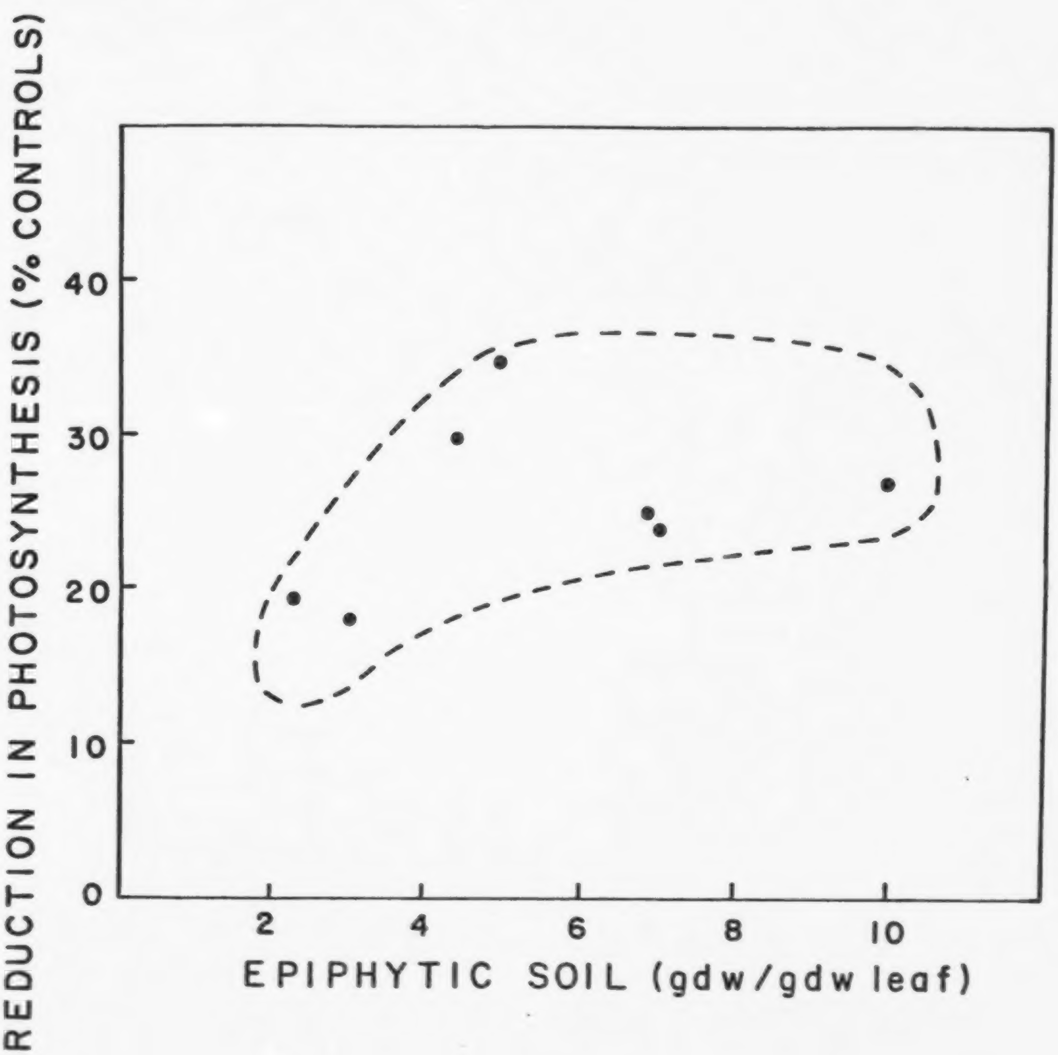

Figure 4. Photosynthetic reduction in leaves of *P. perfoliatus* due to increasing amounts of epiphytic soil.

REFERENCES

1. Armstrong, D.E. and G. Chesters. 1968. Adsorption catalyzed chemical hydrolysis of atrazine. Environ. Sci. Technol. 2:683-689.

2. Borum, J. and S. Wium-Andersen. 1980. Biomass and production of epiphytes on eelgrass (Zostera marinum L.) in the Øresund, Denmark. Ophelia 1:57-64.

3. Correll, D.L., Pierce, J.W., and T.L. Wu. 1978. Herbicides and submerged plants in the Chesapeake Bay. Pages 858-877 in Proc. Symp. on Technical, Environmental, Socio-economical, and Regulatory Aspects of Coastal Zone Management. Am. Soc. Av. Eng.

4. Correll, D.L. and T. Wu. 1982. Aquatic toxicity to submerged vascular plants in simulated estuarine microcosms. Aquatic Botany (In press).

5. Frank, R., and G.J. Sirons. 1979. Atrazine: It's use in corn production and its loss to stream waters in southern Ontario, 1975-1977. The Science of the Total Environment. 12:223-239.

6. Frank, R., G.J. Sirons, R.L., Thomas, K. McMillan. 1979. Triazine residues in suspended soilds (1974-1976) and water (1977) from the mouths of Canadian streams flowing into the Great Lakes. Internat. Assoc. Great Lakes Res. 5(2):131-138.

7. Hershner, C., Ward, K., and J. Illowsky. 1981. The effects of atrazine on Zostera marina in the Chesapeake Bay, Virginia. Interior report, EPA contract #R805953, Annapolis, MD. 169 pp.

8. Laskowski, D.A., and P.J. McCall. 1980. Determination of sorption constants in soil and sediments. (Unpublished manuscript).

9. Means, J.C., T.W., Jones, T.S. Pait, R.D. Wijayaratne. 1981. Adsorption of atrazine on Chesapeake Bay sediments and selected soils. In: Submerged aquatic vegetation in Chesapeake Bay: Its ecological role in bay ecosystems and factors leading to its decline. Kemp, W.M., J.C. Stevenson, W. Boynton, and J.C. Means (Eds.) Submitted to Chesapeake Bay Program USEPA, Annapolis, Maryland. Ref. #UMCEES 81-28 HPEL.

10. Muir, D.C.G., J.Y. Yoo, B.E. Baker. 1978. Residues of atrazine and N-deethylated atrazine in water from five agricultural watersheds in Quebec. Arch. Environm. Contam. Toxicol. 7:221-235.

11. Phillips, G.L., Eminson, D., and B. Moss. 1978. A mechanism to account for macorphyte decline in progressively eutrophicated freshwters. Aquatic Bot. 4:103-126.

12. Sand-Jensen, K. 1977. Effect of epiphytes on eelgrass photosynthesis. Aquatic Bot. 3:55-63.

13. Stevenson, J.C. and N.M. Confer. 1978. Summary of available information on Chesapeake Bay submerged vegetation. U.S. Dept. of Interior.

14. Talbert, R.E., and O.H. Fletchall. 1965. The adsorption of some s-atrazines in soils. Weeds 13:46-52.

15. Triplett, G.B., Jr., B.J. Conner, W.M. Edwards. 1978. Transport of atrazine and simazien in runoff from conventional and no-tillage corn. J. Environ. Qual. 7(1):77-84.

16. Twilley, R.R., W.M. Kemp, K.W. Staver, W.R. Boynton, J.C. Stevenson. 1982. Effects of nutrient enrichment in experimental estuarine ponds containing submerged vascular plant communities. EPA Report, Grant No. R805932-01-1, Annapolis, Md.

17. Wauchope, R.D. 1978. The pesticide content of surface water draining from agricultural fields - A review. J. Environ. Qual. 7(4):459-472.

18. Wauchope, R.D., and R.A. Leonard. 1980. Maximum pesticide concentrations in agricultural runoff: A semiempirical prediction formula. J. Environ. Qual. 9(4):665-672.

19. Wu, T.L. 1980. Dissipation of the herbicides atrazine and alachlor in a Maryland corn field. J. Environ. Qual. 9(3):459-465.

CHAPTER VI

DEGRADATION OF ATRAZINE IN ESTUARINE WATER/SEDIMENT SYSTEMS AND SOILS[1]

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ABSTRACT

Herbicides have been postulated as a cause of the disappearance of submerged aquatic vegetation in the Chesapeake Bay. This research was undertaken to determine the longevity of 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) in two estuarine water/sediment microcosm systems and two agricultural soil systems over an 80-day period under aerobic and low-oxygen conditions. Atrazine degradation proceeded more rapidly in the estuarine systems than in the soil systems. The disappearance of atrazine from the estuarine water was relatively rapid with the half-life (50% remaining in the water column) of the parent compound ranging from 3-12 days. Atrazine half-life was determined to be 15 and 20 days for the two estuarine sediments and 330 and 385 days for the two agricultural soils. Hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine) was the major short-term metabolite in both the estuarine and terrestrial systems. By the 21st day of the experiment, the % of total extracted residues corresponding to atrazine, and hydroxyatrazine, were: 65, 20 and 10, 85, for the two estuarine systems; and 66, 29, and 93, 5 for the two soil systems. Decreased oxygen levels had little effect on atrazine degradation in the experimental systems. The rapid degradation of atrazine to hydroxyatrazine in estuarine water and sediment indicates a low probability for the accumulation of atrazine in the estuary.

Additional index words: Herbicide, estuarine, decomposition, sorption, half-life, metabolites

INTRODUCTION

Over the last several decades agricultural production in the United States increased largely through the development of efficient cropping methods (26). Among the more important factors contributing to improved agricultural yields, has been the extensive use of herbicides for weed control. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) a compound used to control broadleaf weeds, has found widespread application for corn and sorghum crops since its introduction in 1958, and it is currently being tested for pine plantations (6).

Invariably a fraction of the herbicide applied to agricultural fields is transported to watercourses via surface runoff and groundwater interflow. Atrazine and its metabolites have been observed in freshwater streams contiguous to agricultural land where it was found that from about 0.1% to 3.0% of the atrazine placed on the fields is lost to the aquatic environment (9, 10, 18). Most research concerning herbicide losses from agricultural soils has been conducted at inland sites; however, a few recent studies have focused on the coastal plain. For example, Wu (28) has reported a leakage rate of 1% for atrazine in a Chesapeake Bay watershed, producing aqueous concentrations averaging 17 ppb.

The effect of these herbicides on non-target species of aquatic organisms is of ecological concern. Generally, concentrations of atrazine in the range of those observed in runoff are potentially toxic to a variety of estuarine plants (3, 11, 24). A key factor contributing to the phytotoxicity of a herbicide such as atrazine is its longevity or rate of degradation. Atrazine sorption and degradation is influenced by pH, temperature, and soil moisture, as well as clay and organic content of soils (1, 14, 25). Apparently, atrazine persists in most soil environments for a relatively long duration, with a half-life exceeding one year under some conditions.

While Goswami and Green (8) had suggested that the relatively high pH of estuarine and coastal environments (> 7.0) would cause atrazine degradation to be slowed, little empirical evidence has been available to substantiate this hypothesis. Some preliminary results indicate that degradation of atrazine may proceed quite rapidly in a slightly brackish environment (2). However, their experimental system did not make direct comparison to soil systems.

The purpose of this study was to investigate atrazine sorption and degradation in estuarine water and sediments, as well as terrestrial soils, under a range of environmental conditions in the same experimental design.

MATERIALS AND METHODS

Sediment/Soil Samples

Water and surface sediments (top 30 cm) were collected from inshore locations in early July 1979, to represent two salinity and geographical areas of the Upper Chesapeake Bay region. The Tangier Sound sample, 15 parts per thousand salinity, represented an open bay site while the Choptank Estuary sample, 8 parts per thousand salinity, was hydrographically more protected. The estuarine waters were filtered (0.45 μm) and the estuarine sediments sieved (2 mm) and kept moist at 5 C.

Two soils common to the Eastern Shore watershed of the Bay were collected at the same time for direct comparison of degradation rates in terrestrial environments. Sassafras sandy loam (Typic Hapludult), a well drained upland soil and Mattapex silty loam (Aquic Hapludult), a poorly drained lowland soil, were obtained (top 30 cm) from sites where atrazine had not been applied for over 3 yrs. The physical characteristics of the sediments and soils in this study are listed in Table 1. The soils were also sieved (2 mm) and held at 5 C at their ambient moisture content.

Atrazine Applications and Sampling

For the estuarine samples, 600 g of wet-sediment (40% water, by weight) and 500 ml of water from the respective sampling areas were added to each of 6 flasks (2 ℓ), 3 of which remained open to the atmosphere via a cotton-stoppered glass tube through a rubber stopper (aerobic) and the other 3 sealed with rubber stoppers (low-oxygen). The sediments were treated with uniformly ring-labeled atrazine (50 μCi/mg, 95% purity) by the introduction of a slurry containing 1 g of the respective sediment type and 5 μCi (100 mg) of ^{14}C-atrazine in methanol yielding an initial atrazine concentration in each flask of 0.10 ppm (water and sediment). All the flasks were maintained in a water bath at ambient Choptank River temperatures (12-35°C) in full sunlight starting in mid-July 1979 and ending in late October 1979. At each sampling period (days 1, 7, 14, 21, 28, 45, 59, and 80), 1 ml of water from each flask was passed through a 0.45 m syringe filter directly into a scintillation vial containing 10 ml of Aquasol-2 (New England Nuclear) to determine the ^{14}C activity in the aqueous fraction. The filter was then placed in another scintillation vial with Aquasol-2 to determine the ^{14}C present in the suspended particulate fraction.

A 5 ml water sample was also taken from each flask at each sampling period and extracted twice with a total of 10 ml of chloroform. The chloroform (non-polar) extract and the residual 5 ml of water (polar) were evaporated at room temperature and stored at -5 C until analyzed for the parent compound and its major metabolites.

TABLE 1. Physical and chemical characteristics of sediments and soils.†

	pH	Organic Carbon	Sand	Silt	Clay
		%			
Sediments					
CHOPTANK	5.4	0.55	49	34	17
TANGIER	4.4	0.85	59	28	13
Soils					
SASSAFRAS	5.5	0.91	69	14	17
MATTAPEX	6.4	0.92	16	52	32

†Sediments and soils were seived to 2 mm prior to analysis.

Sediment (2 ± 0.5 g) was removed from each flask at each sampling period using a 2 cm diameter glass corer. The sediment was placed into a glass vial containing 5 ml of 10 % aqueous acetonitrile and stored at -5 C until extracted. Following sampling, each flask was lightly swirled to resuspend the surface sediments.

A small quantity (3 mg) of uniformly ring-labeled ^{14}C-atrazine (150 µci) dissolved in 100 ml of methanol was applied to 600 g subsamples each of both Sassafras and Mattapex soils and the methanol allowed to evaporate while the soils were thoroughly mixed. From these subsamples, 100 g aliquots of each soil was placed into flasks (2 ℓ) containing 300 g of untreated soil, and the flasks shaken vigorously. To simulate natural soil moisture conditions for these two soils, water was added to the samples of Sassafras soil to bring them to 60 percent water-holding capacity (17% water by weight) and to the Mattapex soil to bring them to 100 percent water-holding capacity (50% water, by weight). The atrazine concentrations in the flask were 6.4 and 5.0 ppm (water and soil) for the Sassafras and Mattapex soils, respectively. Three of the flasks for each soil type were maintained under aerobic conditions and three under low-oxygen conditions as described for the estuarine flasks. All flasks were held in full sunlight at ambient air temperature over the same period as the estuarine flasks. The soil temperature reached a maximum of 6°C higher than the external air temperature on the hottest day recorded -- 36°C. At each sampling period (same as estuarine samples), a total of 2 ± 0.5 g of soil were removed from three different locations in each flask and collectively placed in a glass vial containing 5 ml of 10 % aqueous acetonitrile and maintained at -5 C. Flasks were weighed at each sampling period and water added as necessary. The ^{14}C loss due to evolution of $^{14}CO_2$ from metabolized atrazine was not assayed.

Extraction From Sediment and Soil

The sediment and soil samples were refluxed in 20 ml of acetonitrile for 4 h. After refluxing, 1 ml aliquot from each extract was radioassayed for ^{14}C activity as described previously. The remaining acetonitrile was refrigerated at -5 C until further analysis by thin layer chromatography (TLC). To determine extractability, selected sediment/soil samples previously extracted by acetonitrile were combusted using a Harvey oxidizer and the $^{14}CO_2$ trapped in Oxifluor (New England Nuclear) ^{14}C-glucose standards were combusted to establish instrument efficiency.

Thin Layer Chromatographic Analysis of Residues

The dried chloroform and water extract residues were dissolved in 0.2 ml of chloroform and 0.2 ml of methanol, respectively, and spotted on 20 x 20 glass plates with a 250 µm layer of silica gel 60 F-254. The plates were developed to a 15 cm-solvent front in benzene-acetic acid-water (60:40:3, v/v/v). Atrazine, and three of its major degradation products, 2-chloro-4-amino-6-isopropylamino-s-triazine (N-de-ethylated), 2-chloro-4-ethylamino-6-amino-s-triazine (N-de-isopropylated), and 2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine (hydroxyatrazine), were co-chromatographed on each plate. Location of the standard compounds was determined with an ultraviolet lamp (254 nm) and location of the extracted compounds by autoradiography using Kodak X-Omat R XR-5 film with an exposure time of 14 days. Typical R_F values of the standards were 0.90, 0.78, 0.74, and 0.25 for atrazine, N-de-ethylated, N-de-isopropylated, and hydroxyatrazine, respectively (the extracted samples had R_F values slightly less than the standards possibly due to retardation by salts in the extracted samples).

The ^{14}C present in each spot appearing on the film was determined by removing the silica gel from the plate (2 cm diameter) and placing it into 10 ml of Aquasol-2. The areas between the origin and hydroxyatrazine and between hydroxyatrazine and the N-dealkylated compounds were also removed and counted separately. Since the R_F values for the N-de-ethylated and N-de-isopropylated daughter compounds were close (R_F of 0.78 and 0.74, respectively), the silica gel from those areas was combined and termed the "dealkylated products." The acetonitrile extracts of the sediments and soils were concentrated under low heat and spotted onto the TLC plates and processed as above.

RESULTS AND DISCUSSION

^{14}C-Atrazine Disappearance from the Water

Atrazine removal from the water can occur via both sorption to the sediment and degradation in the water to metabolic products which may be sorbed on the sediment (Fig. 1). After atrazine sorption to sediments, the compound could be degraded to metabolic compounds and subsequently desorbed into the water column. This mechanism may be of some importance, since atrazine degrada-tion in sterile, filtered water systems has been shown to be extremely slow (1, 2, 7). The dashed lines in Fig. 1 indicate the suspected lesser significance of atrazine degradation in the water. Considering the relatively short duration of our study period (80 days) and the reported slow ring-cleavage of atrazine (5, 29), the evolution of $^{14}CO_2$ by biological degradation was not monitored.

The removal of ^{14}C-activity from the water in both estuarine systems was a relatively rapid process following first-order kinetics (Fig. 2). The aerobic systems were found to have a significantly faster disappearance of ^{14}C activity from the water than the low-oxygen treatments ($p<0.01$). This significance difference comes about mainly because of the slower removal of the ^{14}C-atrazine metabolites in the anaerobic systems after 21 d. This apparent retardation of atrazine residue sorption to sediments due to low-oxygen levels may be of significance in estuarine environments where the redox potential of surface sediments is low. Slowed sorption of atrazine residues out of the water would increase the residence time of these compounds in the aqueous environment permitting an increased uptake by planktonic organisms.

In addition, disappearance of ^{14}C from the water in the Tangier systems was significantly more rapid than in the Choptank systems ($p<0.01$). The initial removal rates (through day 21) for the 4 systems were 1.05, 1.04, 1.10, and 1.07 %/d for the Choptank aerobic, Choptank low-oxygen, Tangier aerobic, and Tangier low-oxygen systems, respectively. The half-lives of total atrazine residues in the water (based only on ^{14}C activity) were 6 and 8 d for the Tangier aerobic and Tangier low-oxygen systems, respectively, and 17 d

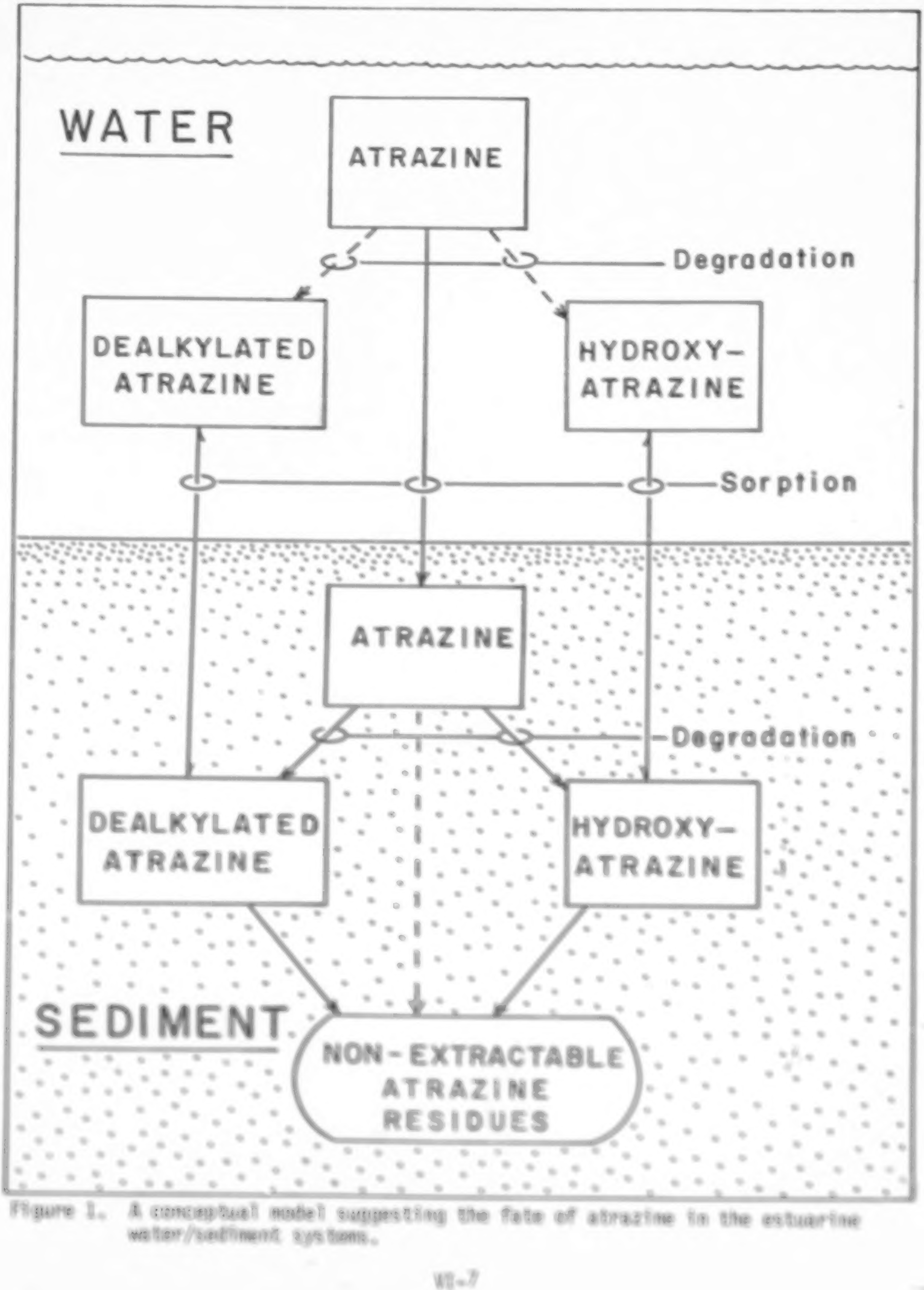
WATER
ATRAZINE
Degradation
DEALKYLATED ATRAZINE
HYDROXY-ATRAZINE
Sorption
ATRAZINE
Degradation
DEALKYLATED ATRAZINE
HYDROXY-ATRAZINE
SEDIMENT
NON-EXTRACTABLE ATRAZINE RESIDUES

Figure 1. A conceptual model suggesting the fate of atrazine in the estuarine water/sediment systems.

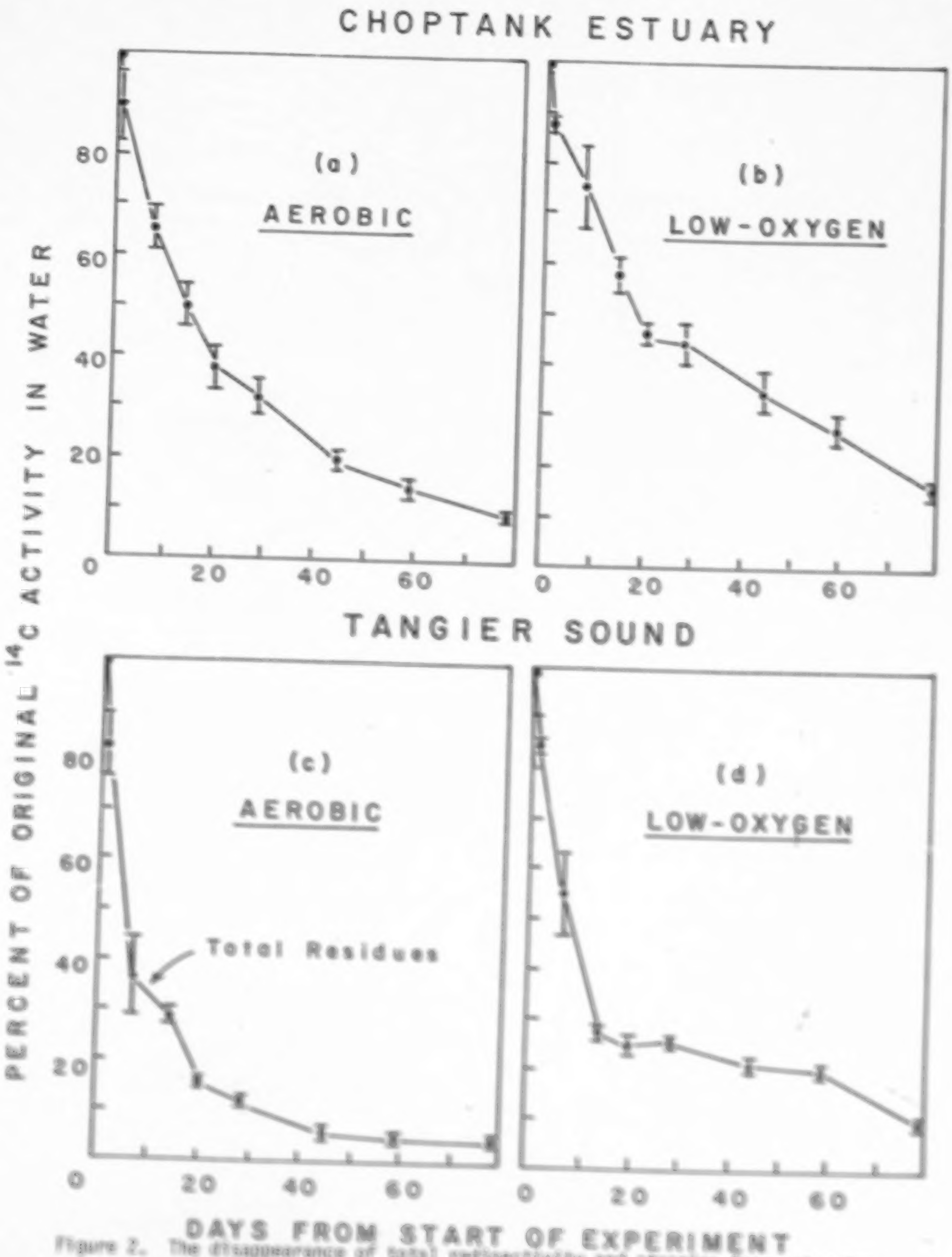

Figure 2. The disappearance of total radioactivity and atrazine from estuarine water.

for the Choptank systems.

These rates of disappearance from the water were much more rapid than those observed by Ballantine et al. (2) in their microcosm study, where they found a half-life of 75 d for total ^{14}C-atrazine residues in the water. The periodic resuspension of the sediment at sampling times and the different water to sediment ratio of the two studies (10:1 in Ballantine et al. vs. 2:1 in this study) may have produced faster disappearance rates. Decreases in the water to soil ratio have been found to lead to increased sorption of atrazine (4).

Atrazine Degradation in Estuarine Water

A strong substrate-product relationship between atrazine and hydroxyatrazine was evident with a low but relatively constant percentage of the ^{14}C activity associated with the dealkylated compounds in both salinity systems (Fig. 3). Unlike the situation discussed above for the total residues (Fig. 2), no significant difference ($p<0.01$) was observed for atrazine degradation under different oxygen levels within salinity systems, but degradation in the Tangier systems occurred significantly faster ($p<0.01$) than in the Choptank systems. The average coefficient of variation for triplicate observations of % atrazine at each sampling time were 19.9, 25.7, 30.3, and 25.0% for the Choptank aerobic, Choptank low-oxygen, Tangier aerobic, and Tangier low-oxygen systems, respectively.

The composition of the total extract only (both chloroform and water residual) is represented by Fig. 3, and does not take into account the total decrease of ^{14}C activity in the water over time (Fig. 2). Therefore, the half-life of atrazine in the water (including both sorption and degradation) was 9 d for the aerobic and 12 d for the low-oxygen Choptank systems, while being about 3 d for both Tangier systems. Again, these rates represent a more rapid disappearance of atrazine from the water than observed by Ballantine et al. (2), where they found the half-life of atrazine in the water to be 30 d.

The observed kinetics of the removal of total ^{14}C from the water and the degradation rate of atrazine in the estuarine systems may result from several associated mechanisms: (1) The rapid hydrolysis of atrazine to hydroxyatrazine in the water linked to the greater sorptive properties of the relatively polar hydroxyatrazine to soil (sediment) (5) would accelerate removal of ^{14}C from the water; and/or (2) ^{14}C-labeled atrazine may be sorbed to sediments initiating a rapid hydrolysis to hydroxyatrazine, whereupon the metabolite desorbs back to the water (see Fig. 1). Catalysis of s-triazines to hydroxytriazine has been shown to occur once the herbicide has been sorbed to clay and organic matter surfaces (1, 20, 22). For example, Armstrong et al. (1) found a 10-fold increase in the rate of atrazine hydrolysis when sterilized soil was added to a solution containing atrazine, while Ballantine et al. (2) detected essentially no atrazine degradation over a 4 mo period in synthetic seawater which contained no sediment. Geller (7) using ^{14}C-atrazine in sterile 0.02 M phosphate buffer (pH 7.2) found less than 5% of the atrazine had degraded after 3 mo.

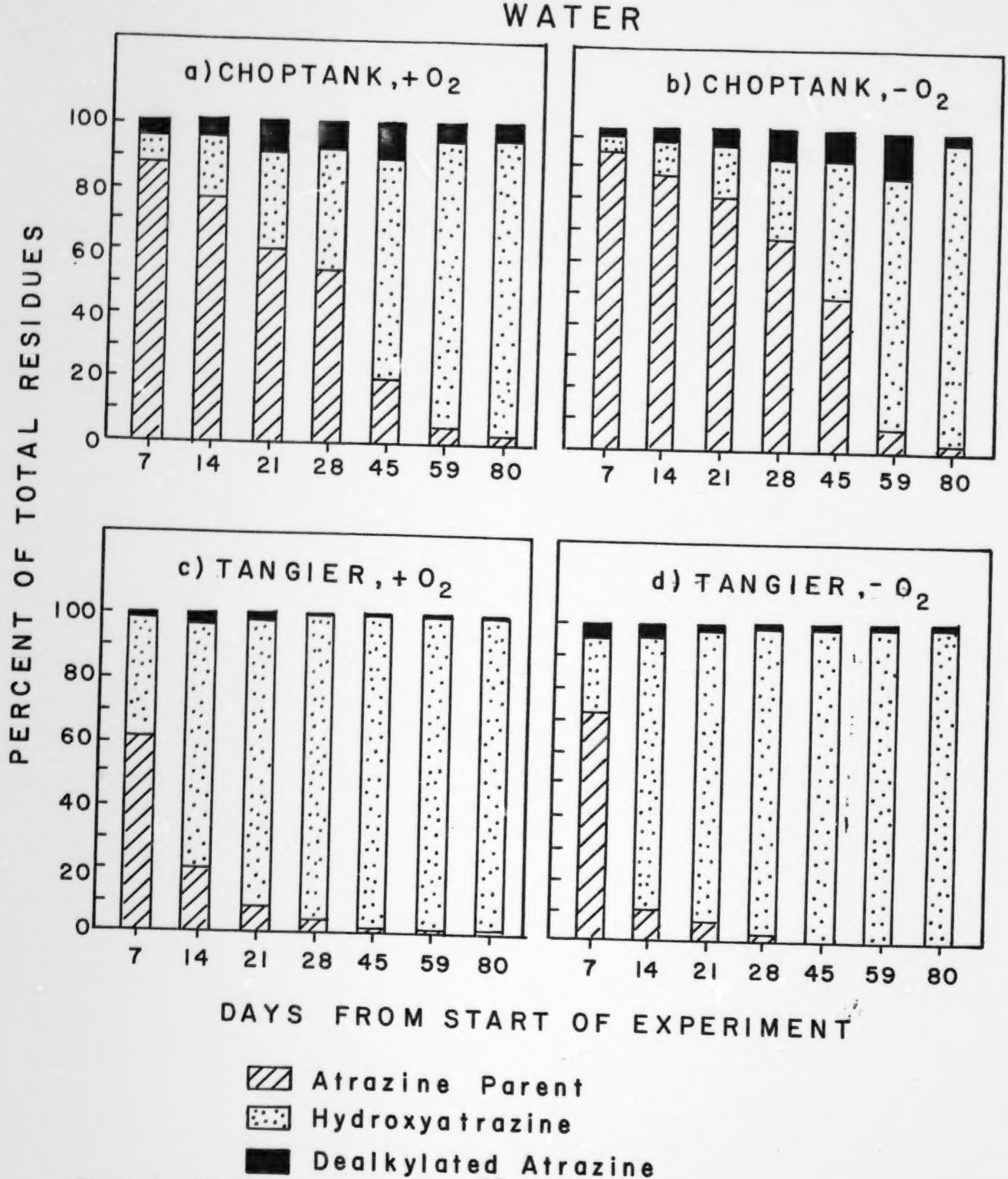

Figure 3. The percent composition of ^{14}C-atrazine metabolites in the water column of the estuarine systems.

Atrazine degradation was most rapid in the Tangier water (Fig. 3) concommittant with the most rapid removal of total ^{14}C from the water (Fig. 2). Conversely, the Choptank water displayed a slower atrazine degradation and sorption rate with a greater percentage of dealkylated products being present. The distribution coefficients [K_d = (μg/g adsorbed)/(μg/ml solution)] of the two sediment types used in the present study were 1.75 and 2.56 for the Choptank and Tangier sediments, respectively (16). The greater adsorptive properties of the Tangier sediment correlates with the observed shorter half-life of atrazine in the Tangier water in relation to the Choptank tending to support the sorption-degradation-desorption mechanism.

Atrazine Degradation in Estuarine Sediment

The % of total ^{14}C-atrazine associated with suspended particulate matter (0.45 μm) in the water proved to be insignificant. The flasks remained undisturbed following sampling allowing particulate matter to settle. Only a very small (initially 10%) quantity of ^{14}C was retained by the filters themselves, and the magnitude of this residue diminished over time as the % of the more polar hydroxyatrazine metabolite increased.

The rate of atrazine degradation and the appearance of the metabolic products in the Tangier and Choptank sediments followed simple, first-order kinetics, with the linearized ($\log_2$ transformed) data producing highly significant ($p<0.01$) regressions having r^2 values in excess of 0.80. The distribution of residues over time followed a pattern similiar to the water (Fig. 4); however, this data represents only the extractable portion of the total radioactivity in the sediment. Extractability decreased over time (Table 2) parallel to the kinetics of atrazine degradation to hydroxyatrazine for each sediment type. For example, the rate of hydroxyatrazine production increased with the % of non-extractable ^{14}C more rapidly in the Tangier than the Choptank sediment. By day 80 of the experiment, only 29% of the total ^{14}C was extractable from the Tangier sediment, of which 90% was hydroxyatrazine (Table 2, Fig. 4). On the same day, 37% of the total ^{14}C was extractable from the Choptank sediment and of this 70% was hydroxyatrazine. The non-extractable ^{14}C atrazine activity most likely consists largely of further degraded metabolites, as well as conjugates with soil organic matter (15). This view is supported by the decrease in % of hydroxyatrazine in the Tangier aerobic system from day 14 (30%) to day 45 (16%) and the concommittant increases in non-extractable activity (57 and 75%, respectively). This suggests that the relative availability of metabolic products for further degradation was greater earlier in the experiment for the Tangier system than for the Choptank. In the combined water/sediment Choptank system, the hydroxyatrazine % increased from day 14 to day 45 (35 to 61%, respectively), re-emphasizing the relatively slower degradation rates of that system.

A similar pattern was reported by Dao et al. (5) for 3 soils held at 0.1 bar moisture content at 30 C. They found that the extraction efficiency ranged from 20 to 40% after 2 mo and that the major portion of the extract was comprised of polar compounds (most likely hydroxyanalogs of atrazine). Their extraction efficiencies compare well to the 30 to 40% extraction efficiency found after 2.5 mo for the sediments in our study (Table 2). Ballantine et al. (2), using a similar extraction procedure as in our study, found that 53% of the radioactivity in the sediment was non-extractable after 1 mo.

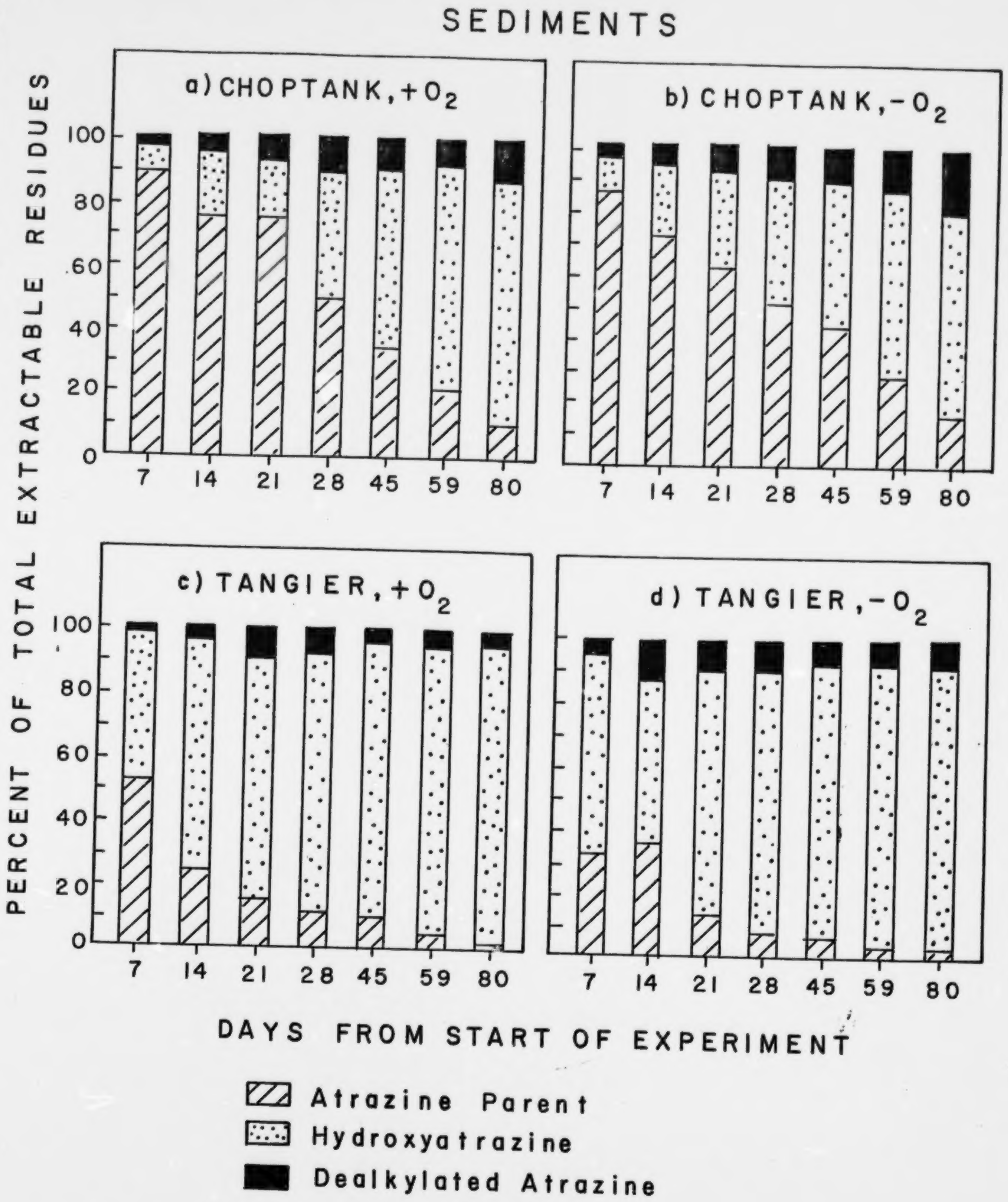

Figure 4. The percent composition of ^{14}C-atrazine metabolites in the total extracted residues from the estuarine sediments.

TABLE 2. Extraction of ^{14}C-atrazine and its metabolites with acetonitrile from sediments and soils.†

Days	Sediment Source		Soil	
	Choptank	Tangier	Sassafras	Mattapex
1	60	50	94	87
14	45	24	83	66
45	28	19	74	35
80	37	29	65	20
108	21	22	23	15

†Data from aerobic samples only, determined by combustion.

A consideration in comparing the results of the Choptank and Tangier systems is that they may contain very different populations of microorganisms with different atrazine degradation potential. The effect that environmental factors might have on the distribution of fungi and bacteria, the major biological degraders of atrazine in the soil (12, 13, 29), is not clear at this time. Several fungal species isolated from the Chesapeake Bay may degrade atrazine (Speedy, pers. com). Never-the-less, the low concentrations of dealkylated atrazine metabolites and the rapid appearance of hydroxyatrazine would suggest a minor role for microorganisms in the degradation of atrazine (23).

Atrazine Degradation in Soils

The apparent kinetics of atrazine degradation (considering only extractable residues) in the Sassafras sandy loam and Mattapex silty loam soils were similar (Fig. 5) but markedly different from the estuarine sediments. Less atrazine was degraded in the soils compared to the estuarine sediments after 80 days. The extractable percentage of ^{14}C in the soils over time (Table 2) shows that the atrazine metabolites on the Sassafras soils were more loosely bound than those on the Mattapex soil (65% extractable versus 20% extractable at day 80, respectively). If one assumes that the non-extractable activity is something other than the parent compound, atrazine degradation in the Mattapex soil was much more rapid than in the Sassafras.

The two major physical differences between the Sassafras and Mattapex systems were the moisture content and the percent silt and clay (60, 14, 10%, and 100, 52, 32%, respectively). Degradation of atrazine to hydroxyatrazine in the Matttapex soil most probably would have been slowed by the high moisture content. High soil moisture content may promote ring-cleavage of atrazine (19) but may also retard the conversion of atrazine to hydroxyatrazine due to reduced adsorption onto the soil (4) and subsequent hydrolysis (1). Conversely, the high clay content in the Mattapex soil may have accelerated atrazine hydrolysis due to sorption on the highly acidic clay surfaces (21, 27). However, the exact source for the difference in degradation rates between the Sassafras and Mattapex soils cannot be determined from the current data.

The issue of extractability has unfortunately received too little attention in the herbicide literature. The fate of atrazine can only be accurately determined when the exact compositon of the non-extractable residues is known. The degradation rate of atrazine can vary markedly depending on how the non-extractable portion is considered. The two extreme cases are where (1) the non-extractable portion is considered to be other than the parent compound, and (2) the non-extractable portion contains the same compounds on a percentage basis as the extractable portion (Table 3). Therefore, the range of half-lives for atrazine calculated from the slopes of the first-order plots in the 4 experimental systems of this study were 13-15, 16-20, 110-330, and 36-385 days for the Tangier, Choptank, Sassafras, and Mattapex systems, respectively. The effect is most severe in the case for the agricultural soils, where half-lives may differ by a factor of 10. Regardless of which assumption is used, however, as long as it is employed consistently for all experimental systems, the degradation rates (indicated by the slope of the linearized, first-order decay) were dramatically greater for the estuarine systems than for the agricultural soils.

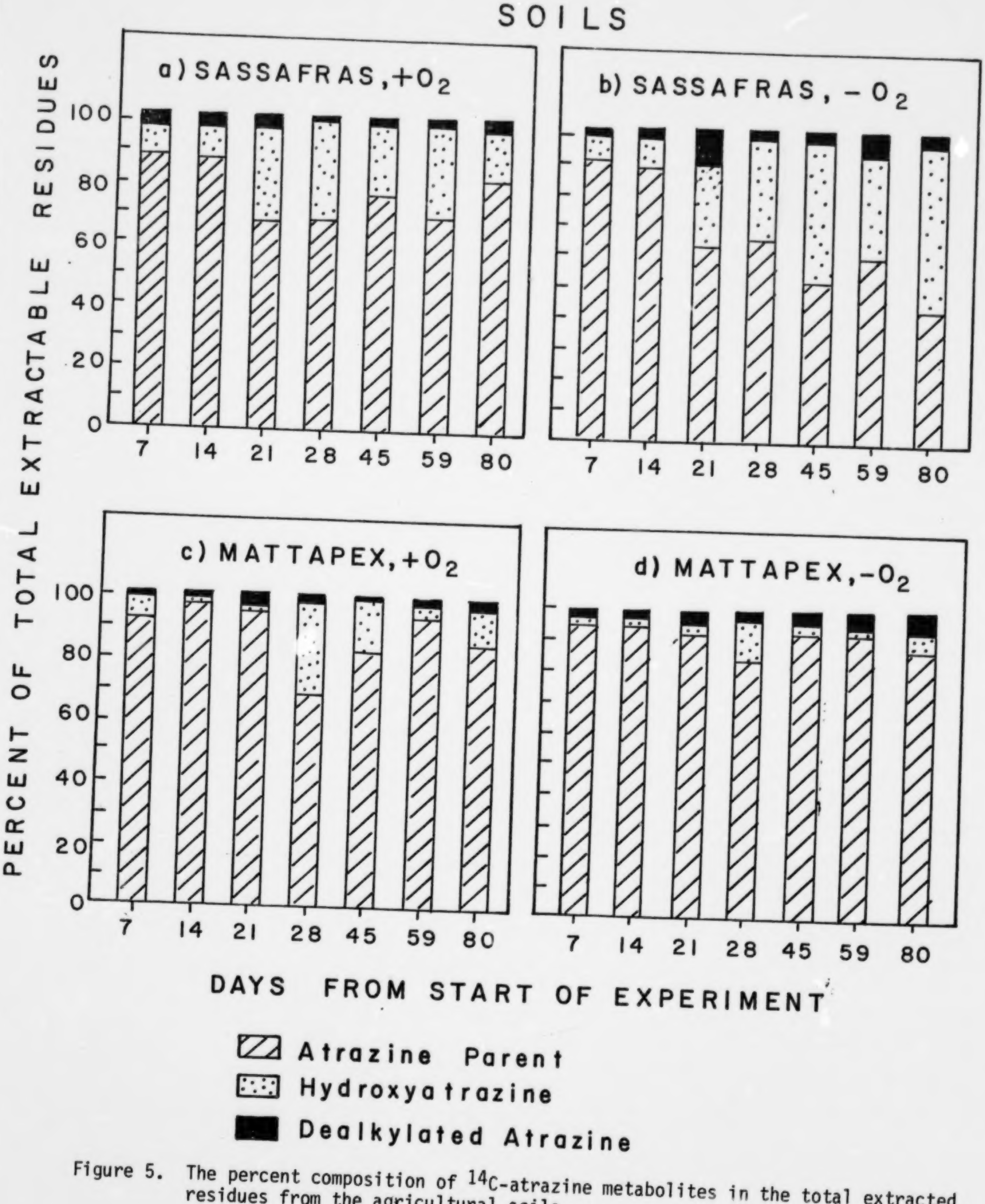

Figure 5. The percent composition of ^{14}C-atrazine metabolites in the total extracted residues from the agricultural soils.

TABLE 3. Atrazine degradation in the aerobic systems with estuarine sediment/water and with agricultural soils under two different assumptions concerning the composition of the non-extractable fraction.

	ATRAZINE REMAINING IN TOTAL SYSTEM†	
	Non-Extractable†† As Non-Atrazine	Non-Extractable††† As All Compounds
Estuarine		
Choptank	%	%
Day 1	86	98
14	49	75
45	9	25
80	3	7
Tangier		
Day 1	71	90
14	5	13
45	2	10
80	1	2
Soil		
Sassafras		
Day 1	88	93
14	73	86
45	56	75
80	53	79
Mattapex		
Day 1	85	96
14	66	96
45	31	82
80	19	86

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† Includes both water and sediment for estuarine systems.

†† Non-extractable radioactivity considered to be compounds other than atrazine.

††† Non-extractable radioactivity considered to contain the same percentage of atrazine as the extractable portion.

The half-life of atrazine in agricultural soils seems to be quite variable with a range of 37 d to as long as 35 y (5, 1, respectively). Less research has been conducted on atrazine degradation in aquatic environments, and therefore, few comparative values are available. However, Armstrong et al. (1) found an atrazine half-life of 145 d in a Wisconsin lake sediment and Ballantine et al. (2) reported a half-life of approximately 30 d for Chesapeake Bay sediment. A general pattern emerges from these data, where degradation rates tend to be substantially slower in agricultural soils (where the half-life generally ranges from 1-6 mo) than in estuarine systems (1-4 wk).

Rapid removal and degradation of atrazine to hydroxyatrazine in estuarine water and sediment as demonstrated in the present study indicates a relatively reduced role of residual phytotoxicity in the estuary for the parent compound. However, the N-dealkylated metabolites (principally N-de-ethylated atrazine) may persist and accumulate in the estuary as has been observed in agricultural fields (5, 17, 22). Although no studies have been conducted on the phytotoxicity of atrazine metabolites to aquatic plants, it is known that N-de-ethylated atrazine is toxic to agricultural crops such as oats while hydroxyatrazine is not (12). Therefore, the ecological impact of these atrazine metabolites in aquatic systems should be examined further.

The non-extractable ^{14}C atrazine activity most likely consists largely of further degraded daughter compounds, as well as conjugates with soil organic matter (20). This view is supported by the decrease in percentage of hydroxyatrazine in the Tangier system from day 14 (30 percent) to day 45 (16 percent) and the concomittant increases in non-extractable activity (57 and 75 percent, respectively). The Tangier system also exhibited more rapid rates of both atrazine removal from the water and parent molecule degradation (Fig. 2). This suggests that the relative availability of daughter products for further degradation was greater earlier on in the experiment in the Tangier system than in the Choptank system. In the combined water/sediment Choptank system, the hydroxyatrazine percentage increased from day 14 to day 45 (35 to 61 percent, respectively), reemphasizing the relatively slower degradation rates of that system.

The half-lives ($T_{0.5}$) of atrazine applied to a variety of soils reported in the literature are provided in Table 4 along with the estuarine and soil systems of the present study for comparative purposes. The problem of non-extractability of atrazine metabolites from soils creates a situation whereby many of the values presented in this table must be considered only approximate. The atrazine half-lives in the soils of the present study are comparable with the reported values from the literature. A general pattern emerges from these data, where degradation rates tend to be substantially slower in agricultural soils (where $T_{0.5}$ generally ranges from 1-6 months) than in estuarine systems (where $T_{0.5}$ ranges from about 1-4 weeks).

In conclusion, it appears that atrazine is generally more labile in the estuarine environment than on agricultural fields. As such, this trend tends to benefit all concerned, since it enhances weed control for the farmer, while diminishing the chances of effects to non-target species in the estuary. However, the N-dealkylated metabolites (principally N-de-ethylated atrazine) may persist and accumulate in the estuary. Moreover, there is some reason to suspect that this compound may retain some phytotoxicity. For example, Sirons et al. (26) found that N-de-ethylated atrazine was more persistent than atrazine in soils, and because of its phytotoxic nature may account for the carry-over of phytotoxicity observed from one year to the next in soils following atrazine applications. Dao et al. (6) found the levels of N-de-ethylated atrazine to remain almost constant in atrazine-treated soils for two years following application. Rapid removal and degradation of atrazine to hydroxyatrazine in estuarine water and sediment as demonstrated in the present study indicates a relatively reduced role of residual phototoxicity in the estuary for the parent compound. However, the relative persistance of the dealkylated daughter products and their unknown phototoxicity to estuarine plants should be examined further.

LITERATURE CITED

1. Armstrong, D.E., C. Chesters and R.F. Harris. 1967. Atrazine hydrolysis in soil. Soil Sci. Soc. Am. Proc. 31:61-66.

2. Ballantine, L.G., L.C. Newby and B.J. Simoneaux. 1978. Fate of atrazine in a marine environment. Fourth International Congress of Pesticide Chemistry, IUPAC, Zurich, Switzerland.

3. Cunningham, J.J. 1979. Responses of microcosm communities containing submerged aquatic vegetation to herbicide stress. Master's thesis, Univ. of Maryland. 63 pp.

4. Dao, T.H. and T.L. Lavy. 1978. Atrazine adsorption on soil as influenced by temperature, moisture content, and electrolyte concentration. Weed Sci. 26:303-308.

5. Dao, T.H., T.L. Lavy and R.C. Sorensen. 1979. Atrazine degradation and residue distribution in soil. Soil Sci. Soc. Am. J. 43:1129-1134.

6. Eckert, R.E. 1979. Establishment of pine (*Pinus* spp.) transplants in perennial grass stands with atrazine. Weed Sci. 27:253-257.

7. Geller, A. 1980. Studies on the degradation of atrazine by bacterial communities enriched from various biotopes. Arch. Environ. Contam. Toxicol. 9:289-305.

8. Goswami, K.P. and R.E. Green. 1971. Microbial degradation of the herbicide atrazine and its 2-hydroxy analog in submerged soils. Environ. Sci. and Tech. 5:426-429.

9. Hall, J.K., M. Pawlus and E.R. Higgins. 1972. Losses of atrazine in runoff water and soil sediment. J. Environ. Qual. 3:172-176.

10. Hormann, W.D., J.C. Tournayre and H. Egli. 1979. Triazine herbicide residues in Central European Streams. J. Pest Monitoring. 13:128-131.

11. Jones, T.W., J.C. Means, J.C. Stevenson, W.M. Kemp. 1981. Uptake and phytotoxicity of atrazine in *Potamogeton perfoliatus*. In: Submerged aquatic vegetation in Chesapeake Bay: Its ecological role in bay ecosystems and factors leading to its decline." Kemp, W.M., J.C. Stevenson, W. Boynton, and J.C. Means, (Eds.) Submitted to Chesapeake Bay Program USEPA, Annapolis, Maryland, Ref. #UMCEES 81-28 HPEL.

12. Kaufman, D.D. and J. Blake. 1970. Degradation of atrazine by soil fungi. Soil Biol. Biochem. 2:73-80.

13. Kaufman, D.D. and P.C. Kearney. 1970. Microbial degradation of triazine herbicides. Residue Rev. 32:235-265.

14. Kells, J.J., C.E. Rieck, R.L. Blevins and W.M. Muir. 1980. Atrazine dissipation as affected by surface pH and tillage. Weed Sci. 28:101-104.

15. Lamoureau, G.L., L.E. Stafford, R.H. Shimabukuro and R.G. Zaylski. 1973. Catabolism of the glutathione conjugate of atrazine. J. Agr. Food Chem. 21:1020-1025.

16. Means, J.C., T.W. Jones, T.S. Pait, R.D. Wijayaratne. 1981. Adsorption of atrazine on Chesapeake Bay sediments and selected soils. In: Submerged aquatic vegetation in Chesapeake Bay: Its ecological role in bay ecosystems and factors leading to its decline." Kemp, W.M., J.C. Stevenson, W. Boynton, and J.C. Means (Eds.) Submitted to Chesapeake Bay Program USEPA, Annapolis, Maryland. Ref. #UMCEES 81-28 HPEL.

17. Muir, D.C.G. and B.E. Baker. 1978. The disappearance and movement of three triazine herbicides and several of their degradation products in soil under field conditions. Weed Res. 18:111-120.

18. Muir, D.C.G., J.Y. Yoo and B.E. Baker. 1978. Residues of atrazine and N-de-ethylated atrazine in water from five agricultural watersheds in Quebec. Arch. Environ. Contam. Toxicol. 7:221-224.

19. Obien, S.R. and R.E. Green. 1969. Degradation of atrazine in four Hawaiian soils. Weed Sci. 17:509-514.

20. Roeth, F.W., Lavy, T.L., and O.C. Burnside. 1969. Atrazine degradation in two soil profiles. Weed Sci. 17:202-205.

21. Russell, J.D., M. Cruz, J.L. White, G.W. Bailey, W.R. Payne, Jr., J.D. Pope, Jr., and J.J. Teasley. 1968. Mode of chemical degradation of s-triazine by montimorillonite. Science. 160:1340-1342.

22. Sirons, G.R., R. Frank and T. Sawyer. 1973. Residues of atrazine cyanazine and their phytotoxic metabolites in a clay loam soil. J. Agric. Food Chem. 21:1016-1020.

23. Skipper, H.D. and V.V. Volk. 1972. Biological and chemical degradation of atrazine in soils. Weed Sci. 20:344-347.

24. Stevenson, J.C. and N.M. Confer. 1978. Summary of available information on Chesapeake Bay submerged vegetation. Fish and Wildlife Service, Publ. OBS-78/66. U.S. Dept. of the Interior.

25. Swanson, R.A. and G.R. Dutt. 1973. Chemical and physical processes that affect atrazine and distribution in soil systems. Soil Sci. Soc. Amer. Proc. 37:872-876.

26. U.S. Department of Commerce. 1974. Census of agriculture. GPO, Washington, D.C.

27. White, J.L. 1975. Determination of susceptibility of s-triazine herbicides to protonation and hydrolysis by mineral surfaces. Arch. Environ. Contam. Toxicol. 3:461-469.

28. Wu, T.L. 1980. Dissipation of the herbicides atrazine and alachlor in a Maryland corn field. J. Environ. Qual. 9:459-465.

29. Wolf, D.C. and J.P. Martin. 1975. Microbial decomposition of ring-^{14}C atrazine, cyanuric acid, and 2-chloro-4,6-diamino-s-triazine. J. Environ. Qual. 4:134-139.

CHAPTER VII

UPTAKE AND PHOTOSYNTHETIC INHIBITION OF ATRAZINE AND ITS DEGRADATION PRODUCTS ON FOUR SPECIES OF SUBMERGED VASCULAR PLANTS[1]

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ABSTRACT

The photosynthetic inhibitory effects of atrazine and three of its major metabolites, deethylated, deisopropylated, and hydroxyatrazine, was determined for four species of submerged macrophytes. All species showed a similar response to varied dosages of the parent atrazine compound with an average I_1 (concentration at which photosynthesis is inhibited by 1%) for all plants of 20 ppb and an average I_{50} (concentration at which photosynthesis is inhibited by 50%) for all plants of 95 ppb. The degradation metabolites of atrazine produced varying degrees of photosynthetic inhibition in the four species, but generally the order of toxicity was deethylated > deisopropylated > hydroxyatrazine with hydroxyatrazine causing an apparent stimulation of photosynthesis in several species. Of four species tested *Myriophyllum spicatum* was the most resistant to atrazine and its metabolites. The magnitude of the actual uptake of the compounds (µg compound/gdw plant) by the plants correlated closely with the photosynthetic inhibitory response, i.e., at the same concentration the uptake of atrazine > deethylated > deisopropylated > hydroxyatrazine. Considering that an extremely high environmental concentration (0.5 ppm) of deethylated atrazine for an estuary only produced a photosynthetic inhibition of from 20 to 40% in four major species of submerged macrophytes, it is concluded that the degradation products of atrazine tested are not of major significance in the disappearance of the submerged grasses from the Chesapeake Bay.

INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), a compound used to control broadleaf weeds, has found widespread application for corn and sorghum crops since its introduction in 1958. Currently, it is the most widely used herbicide in the Chesapeake Bay watershed (Stevenson and Confer 1978). Recent studies concerning runoff from cropland in the Chesapeake Bay watershed where atrazine has been applied have shown that a measurable percent (ca 1%) of the herbicide is ultimately transported from agricultural fields to the aquatic environment (Wu 1980). Thus, herbicides such as atrazine represent a potential source of stress for the vegetation in aquatic environments (e.g., estuaries) and these compounds have been suggested as a possible cause for the recent decline of submerged aquatic vegetation (SAV) in Chesapeake Bay (Correll et al. 1978). Dissolved concentrations of atrazine up to 17 ppb were found by Correll and Wu (1981) and as high as 100 ppb by Kemp et al. (1982) in agricultural runoff, while concentrations of as low as 5-10 ppb have been shown to significantly reduce photosynthesis in various SAV species (Kemp et al. 1982, Forney and Davis 1981, Jones et al. 1982).

A point of ecological concern in herbicide studies is the degradation rate for these compounds in the environment. Atrazine can persist in soil environments for a relatively long duration, with a half-life (time until 50% of the parent compound remains) exceeding one year under some conditions (Armstrong et al. 1967). Degradation has been found to occur much more rapidly, however, under the unique conditions found in shallow estuarine environments, where Jones et al. (1982) found the half-life of atrazine to be approximately 2 weeks.

The degradation of atrazine in agricultural and estuarine environments can occur by several pathways, including (1) 2-hydroxylation forming 2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine(hydroxyatrazine), (2) N dealkylation principally forming 2-chloro-4-amino-6-isopropylamino-striazine (deethylated atrazine) or 2-chloro-4-ethylamino-6-amino-s-triazine (deisopropylated atrazine), or 2-chloro-4,6-diamino-s-triazine, and (3) ring cleavage releasing CO_2 (Armstrong et al. 1967, Kaufman and Blake 1970, Skipper and Volk 1972, Goswami and Green 1971, Wolf and Martin 1975, and Jones et al. 1982). The resulting dealkylated degradation compounds show mixed phytotoxicities for terrestrial plant species (Shimabukuro 1967, Kaufman and Blake 1970, Sirons et al. 1973), while hydroxyatrazine has been shown to have negligible phytotoxic effects (Gysin and Knusli 1960, Kaufman and Blake 1970).

Studies on agricultural soils (Sirons et al. 1973, Dao et al. 1979) as well as in estuarine environments (Jones et al. 1982) have indicated that the atrazine degradation metabolite, deethylated atrazine, may be more persistant than the parent compound and could account for carry-over toxicity in agricultural soils from one year to the next. However, no data exist on the effects of this compound or other atrazine metabolites on any SAV species nor on the uptake of the compounds by these species.

The purpose of this study was to investigate the phytotoxicities of atrazine and its 3 principle degradation metabolites (deethylated atrazine, deisopropylated atrazine, and hydroxyatrazine), and the magnitude of their uptake by 4 important SAV species found in Chesapeake Bay.

METHODS & MATERIALS

Phytotoxicity of Atrazine and its Metabolites

Four submerged aquatic plant species, *Potamogeton perfoliatus*, *Ruppia maritima*, *Myriophyllum spicatum* and *Zannichellia palustris* were collected from the Choptank River estuary and its tributaries (8-12 ppt salinity). Freshly collected plant shoots were rinsed in filtered (1 μm) Choptank water to remove epiphytes and sediment, and cut to a specified length (10-15 cm depending on species). Each plant was placed in an individual Wheaton 300 mℓ BOD bottle containing filtered Choptank water and a predetermined concentration of either atrazine or one of its 3 principle degradation products; deethylated atrazine, deisopropylated atrazine, or hydroxyatrazine. Stock solutions of atrazine and its metabolites were prepared by dissolving the compounds in methanol such that a small volume (1-3 mℓ) could be added to 1500 mℓ of filtered water to achieve the desired concentrations and this water used to fill the BOD bottles.

The experimental design consisted of an array of 5 replicates each of atrazine, deethylated, deisopropylated and hydroxyatrazine. Five replicate controls and dark bottles were incubated simultaneously as well as 5 methanol control bottles which contained a volume of methanol equal to that introduced into the experimental bottles with the herbicide. Duplicate 2 hour incubations were carried out at herbicide concentrations of 0.5 ppm, 1.0 ppm, and 1.5 ppm in water baths maintained at 23 ± 2°C. Light was provided by a fluorescent light bank yielding an intensity of 180 μEin/m^2/sec at the bottle surfaces. Oxygen levels were measured with an Orbisphere Laboratories Oxygen Indicator Model 2607. Following the final oxygen reading each plant was removed and dried to a constant weight at 60°C. Oxygen production was calculated as O_2 ppm/g dry wt/hr. The same experimental design was also employed to test the effects of lower concentrations of atrazine only (10, 25, 50, 100, 250 ppb), in place of the atrazine metabolite compounds. All other conditions remained the same.

Uptake of ^{14}C Atrazine Metabolites

Plants of the same 4 species as above were collected in the previously described manner and placed in separate 2 ℓ erhlenmyer flasks containing 1 ℓ of filtered river water and 0.5 ppm of either ^{14}C ring-labelled atrazine (specific activity (s.a.) 50 μci/mg), deethylated atrazine (s.a. 21.3 μci/mg), deisopropylated atrazine (s.a. 13.7 µci/mg), or hydroxyatrazine (s.a. 33 µci/mg).

Incubations were conducted at 28°C in the light. After 2 hours the plants were removed, rinsed in filtered Choptank water, dried at 60°C and ground with a mortar and pestle. Duplicate samples were processed basically according to the method of Beer et al. (1982). A 15 mg subsample was taken from each ground plant sample and placed in a vial to which 0.1 mℓ water and 1 mℓ Beckman BTS-450 tissue solubilizer was added. The sample was digested at 60°C for 24 hours, after which time 1 mℓ was transferred to a vial containing 10 mℓ Beckman Ready-Solv NA pre-mixed liquid scintillation cocktail. All samples were counted on a Packard Tri-Carb 460C liquid scintillation counter. The external standard ratios method was used to determine counting efficiency.

RESULTS AND DISCUSSION

Atrazine Toxcity

All four species of SAV responded in a similar manner to the dosage levels (10-250 ppb) of atrazine tested, and analysis of covariance revealed no significant differences ($P<.01$) in dose-response pattern among species. A representative log plot is given in Figure 1, where the percent photosynthetic inhibition caused by increasing atrazine concentrations is shown for Ruppia maritima. Although some photosynthetic inhibition was evident in Zannichellia and Myriophyllum at 25 ppb atrazine, significant effects did not appear until 50 ppb for all four species (Table 1). Values of I_1 (concentration at which photosynthesis is inhibited by 1%) and I_{50} (concentration at which photosynthesis is inhibited by 50%) calculated from the linear regression equations for the four species are presented in Table 2. The species were very similar in their I_1 values (~ 20 ppb) indicating that they all have approximately the same threshold sensitivity to atrazine. However, the I_{50} values were slightly more variable among the four species with Potamogeton being the most sensitive (77 ppb) and Myriophyllum being the most resistant (104 ppb).

These values are in close agreement with the findings of two other studies working with Potamogeton and Myriophyllum where oxygen or the ^{14}C-technique was used to assess short-term (2-4 h) photosynthesis (Kemp et al. 1982, Jones et al. 1982). Kemp et al. (1982) in their atrazine study on Potamogeton also monitored biomass and found an I_{50} of 130 ppb. Although Forney and Davis (1981) using stem length or dry weight as the plant response to atrazine found similiar I_1 values as in our study, they found quite different I_{50} values. For Potamogeton they reported an I_{50} for length of 474 ppb and for Myriophyllum, 1104 ppb. The variations in results of the effect of atrazine on such growth responses as length and dry weight in aquatic macrophytes is not surprising when considering the difficulty in maintaining these plants under laboratory conditions for extended periods of time (e.g., Correll et al. 1978). It seems that the most consistent results have been obtained using short-term experiments monitoring photosynthetic parameters as measures of toxicity. However, these results may not be the most revealing as to the total biological effect of atrazine on the plants populations in their environment, and hence there is a

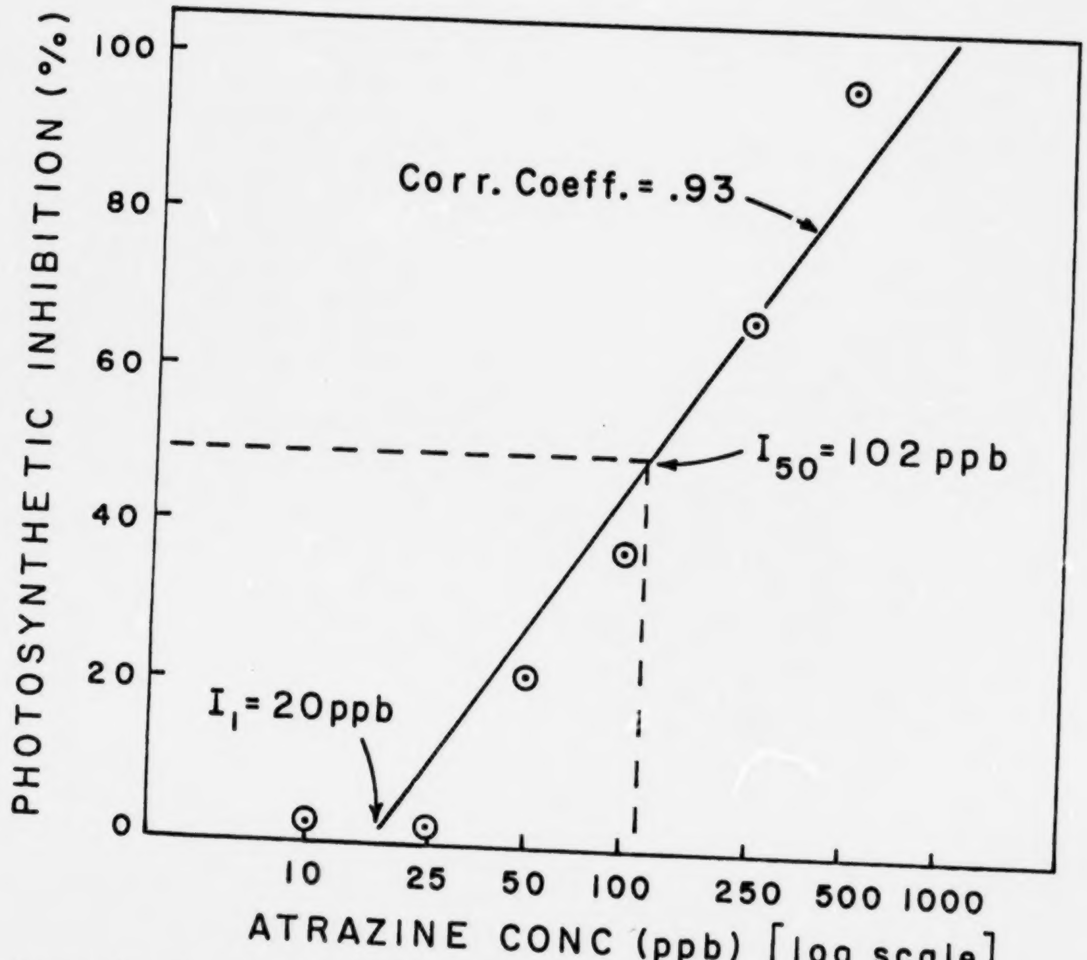

Fig. 1. Linear regression of atrazine effects on photosynthesis in Ruppia maritima indicating calculated I_1 and I_{50} concentrations.

TABLE 1. Apparent oxygen production (mg O_2 gdw^{-1}h^{-1}) of plants exposed to atrazine.*

Atrazine Treatment	Plant Species			
	Zannichellia palustris	*Ruppia maritima*	*Myriophyllum spicatum*	*Potamogeton perfoliatus*
Control	6.46^{a}	8.93^{a}	15.44^{a}	18.12^{a}
10 ppb	6.55^{a}	8.75^{a}	15.43^{a}	17.09^{a}
25 ppb	5.97^{a}	8.79^{a}	15.02^{a}	18.42^{a}
50 ppb	4.84^{b}	6.99^{b}	11.88^{b}	13.01^{b}
100 ppb	2.53^{c}	5.61^{b}	9.56^{b}	8.78^{c}
250 ppb	1.78^{c}	2.96^{c}	4.44^{c}	3.19^{d}

*Given are mean values for 10 replicates followed by a letter designating statistically different groups for a given species ($p<0.05$) using the Student-Neuman-Keuls test.

TABLE 2. Calculated values of I_1 and I_{50} (ppb) as derived from linear regression analysis of % photosynthetic inhibition versus log of atrazine concentration.*

Plant Species	I_1	I_{50}	Corr Coef
Potamogeton perfoliatus	20	77	.93
Myriophyllum spicatum	20	104	.95
Zannichellia palustris	17	91	.95
Ruppia maritima	20	102	.93

*I_1 and I_{50} are defined as the atrazine concentration at which 1% and 50% photosynthetic inhibition are apparent, respectively, (c.a., Forney and Davis 1980).

need for the longer duration experiments.

Atrazine Metabolite Toxicities

The repsonses of the four species of SAV tested to three levels (0.5, 1.0, 1.5 ppm) of atrazine and its 3 metabolites are shown in Figure 2. All four plants had similar photosynthetic responses to the degradation products; however, several significant differences between species is evident. *Myriophyllum* was less affected by the degradation products relative to the other species. Also, increasing concentrations of deisopropylated and deethylated atrazine did not produce concommitant increases in photosynthetic inhibition in *Myriophyllum*, possibly indicating the approach of saturation levels of these compounds in reducing photosynthesis in this species. These results reinforce the observation that *Myriophyllum* is relatively resistant to atrazine as presented above (also see Kemp et al. 1982) and may indicate that this species is able to detoxify atrazine to some extent. Also the four species displayed varying responses to hydroxyatrazine in a range from photosynthetic inhibition at all 3 concentrations (*Zannichellia*) to a stimulation of photosynthesis at all concentrations (*Potamogeton*).

The actual oxygen production rates by the four species exposed to atrazine and its metabolites are presented in Table 3, indicating significant differences ($P<.05$) between the means. Hydroxyatrazine had no significant effect on any of the plants at any of the three concentrations tested. Deisopropylated atrazine exhibited mixed effects in the four species at concentrations of 0.5 and 1.0 ppm and only significantly depressed photosynthesis in all species at the 1.5 ppm concentration. Deethylated atrazine had a significant effect at all concentrations in all plants as did the parent compound.

Atrazine Metabolite Uptake

The magnitude of the uptake of atrazine and its metabolites in the tissue of the four species (Fig. 3) can be related very closely to the relative polarities of the molecules. Relative polarity decreases in the order; hydroxyatrazine > deisopropylated atrazine > deethylated > atrazine. The large lipoid component of the chlorophyllous tissue in these plants would enhance atrazine uptake and retard the movement of the more polar compounds especially hydroxyatrazine into the plant. This mechanism of selective herbicide penetration was proposed earlier by Shimabukuro and Swanson (1969) for atrazine, but, no data were presented. The data presented here may support such a mechanism.

However, when the magnitude of photosynthetic inhibition (% of control) is compared to the uptake of each compound (µg/gdw plant) (Fig. 4) an indication of a more complex situation emerges. For example, the uptake of deethylated atrazine by *Zannichellia* was nearly as great as the uptake of atrazine (10 µg/gdw vs 11 µg/gdw, respectively) and yet the photosynthetic inhibition in response to this uptake was dramatically different. Atrazine reduced photosynthesis by 85% while deethylated atrazine created a reduction of only 40%. A similar situation occurred in *Potamogeton* and to a lesser extent *Ruppia*. Only *Myriophyllum* had a close relationship between uptake and photosynthetic inhibition.

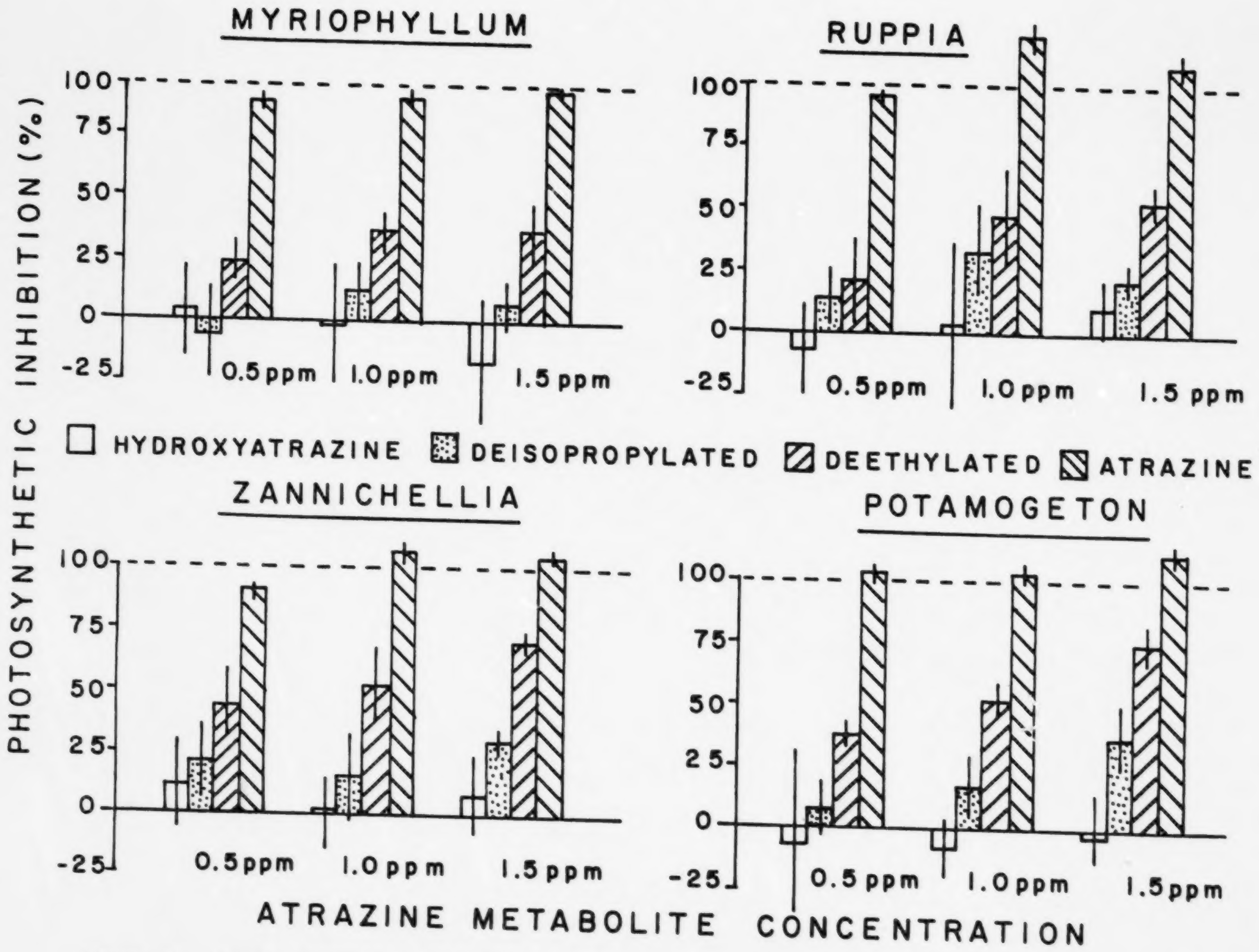

Figure 2. Atrazine metabolite effects on net photosynthesis for the four species at concentrations of 0.5, 1.0, and 1.5 ppm ($\bar{x}$ ± S.D., n = 4).

TABLE 3. Apparent oxygen production (mg O_2 $gdw^{-1}h^{-1}$) of plants exposed to three levels of atrazine and its metabolites.

		Plant Species			
Concentration	Treatment[†]	*Zannichellia palustris*	*Ruppia maritima*	*Myriophyllum spicatum*	*Potamogeton perfoliatus*
0.5 ppm	Control	59.18^{a}[*]	44.45^{a}	51.76^{a}	54.93^{a}
	OH	52.95^{a,b}	42.35^{a,b}	50.29^{a}	52.23^{a}
	Deiso	48.64^{b}	37.82^{b}	54.55^{a}	48.92^{a}
	Deethyl	36.85^{c}	41.04^{b}	41.04^{b}	36.47^{b}
	Atrazine	12.23^{d}	10.67^{c}	10.72^{c}	8.26^{c}
1.0 ppm	Control	63.93^{a}	40.16^{a}	72.92^{a}	58.15^{a}
	OH	63.72^{a}	39.36^{a}	73.98^{a}	61.80^{a}
	Deiso	56.14^{a}	30.99^{b}	64.88^{a}	49.91^{b}
	Deethyl	36.96^{b}	26.92^{b}	49.46^{b}	31.79^{c}
	Atrazine	8.73^{c}	6.21^{c}	11.71^{c}	6.08^{d}
1.5 ppm	Control	61.06^{a}	37.97^{a}	54.59^{a,b}	68.87^{a}
	OH	57.15^{a,b}	34.84^{a,b}	62.50^{a}	69.88^{a}
	Deiso	45.68^{b}	30.90^{b}	51.15^{b}	48.08^{b}
	Deethyl	25.02^{c}	20.97^{c}	37.03^{c}	26.26^{c}
	Atrazine	7.47^{d}	3.53^{d}	7.22^{d}	5.83^{d}

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[*] Given are mean values for 10 replicates followed by a letter designating statistically different groups for a given species ($p \leq 0.05$) using the Student-Neuman-Keuls test.

[†] Treatments are control, hydroxyatrazine (OH), deisopropylated atrazine (Deiso), deethylated atrazine (Deethyl), and atrazine parent compound.

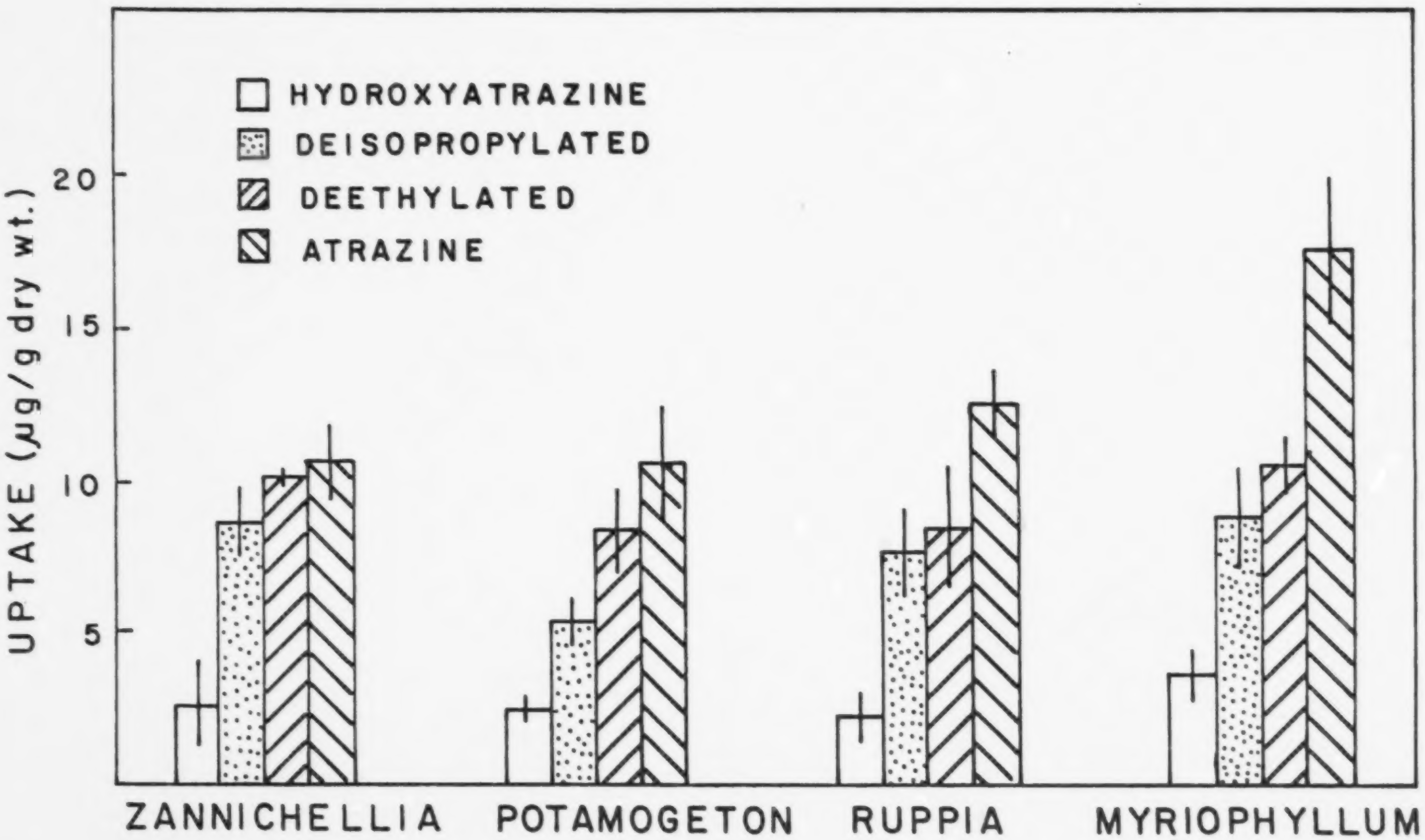

Figure 3. Atrazine metabolite uptake by the four species at a solution concentration of 0.5 ppm for each metabolite ($\bar{x}$ ± S.D., n = 4).

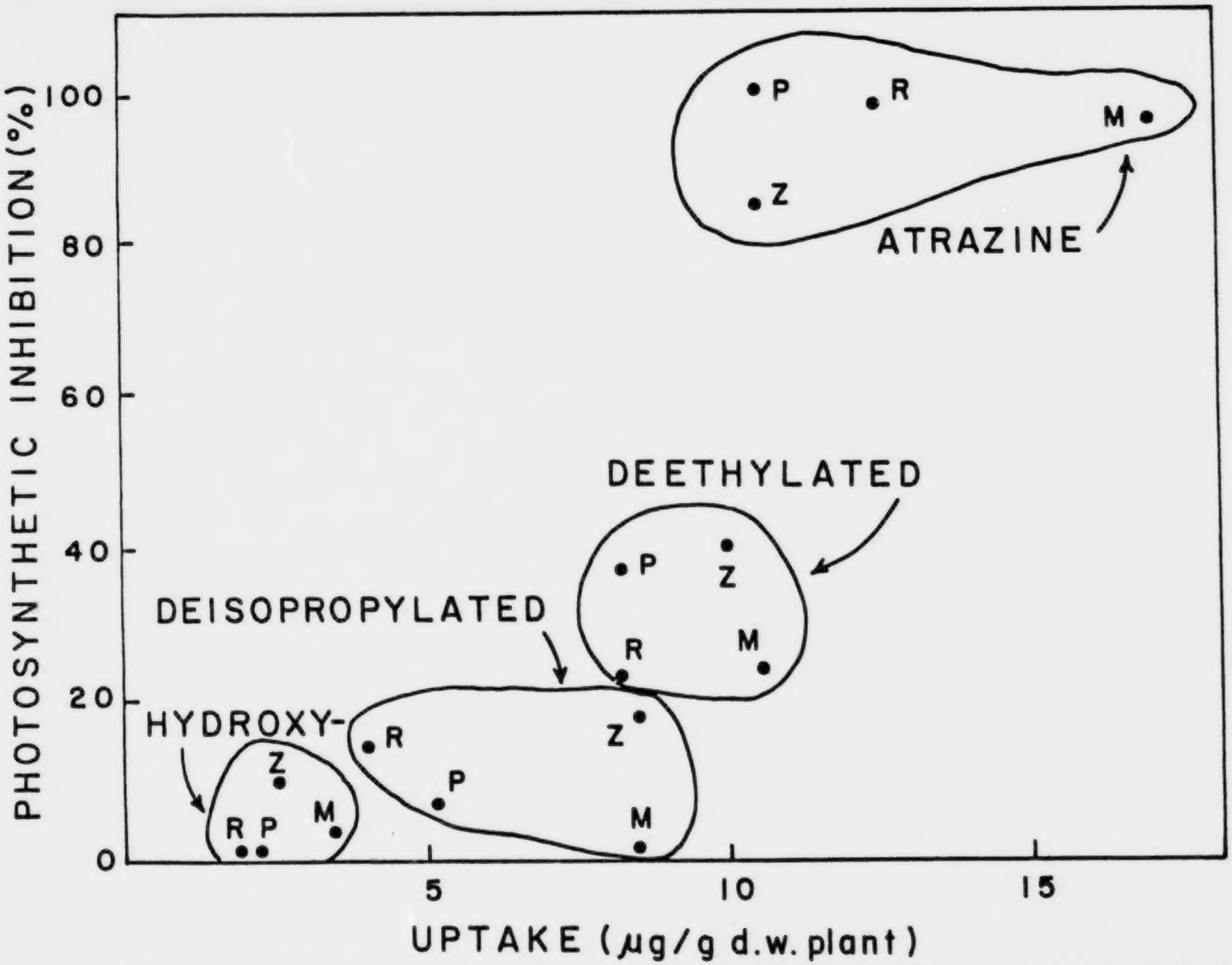

Figure 4. Relationship between uptake of atrazine metabolites and photosynthetic inhibition in the four species at a solution concentration of 0.5 ppm for each metabolite.

The observations reported above may indicate not only a solubility factor in the mechanism of atrazine toxicity, but also possibly steric factors as discussed elsewhere in detail for s-triazines (Ebert and Dumford 1976). The current understanding of the mechanism of action of atrazine is that it binds to a specific protein in the thylakoid membrane (Pfister et al., 1980) which requires that the compounds possess the appropriate configuration. The removal of groups from the s-triazine ring or hydroxylation of atrazine to form degraded metabolites such as those tested here would create steric changes. Pfister et al. (1979) has further proposed a resistance mechanism where plants modify the herbicide binding site such that the compound cannot bind and thus produces no effect. The percent photosynthetic inhibition per µg deethylated atrazine taken up by the plant was only 1/2 that for the same amount of atrazine absorbed. Therefore, the compound was present in the cells but less effectual in reducing photosynthesis compared to atrazine. However the location of the compound is most important for it still could be that the compound is unable to penetrate the chloroplasts due to polarity, and thus it remains chiefly in the cytosol.

It would appear that the atrazine degradation metabolites tested here are not as effectual in reducing photosynthesis in SAV species as atrazine is in a system such as the Chesapeake Bay. The high concentration (0.5-1.5 ppm) of the degradation products tested were necessary to ellicit detectable responses and are far above the concentrations that would be expected in the environment.

REFERENCES

1. Armstrong, D.E., C. Chesters, and R.F. Harris. 1967. Atrazine hydrolysis in soil. Soil Sci. Soc. Am. Proc. 31:61-66.

2. Beer, S., Stewart, A.J., and R.G. Wetzel. 1982. Measuring chlorophyll a and ^{14}C-labeled photosynthate in aquatic angiosperms by the use of a tissue solubilizer. Plant Physiol. 69:54-57.

3. Correll, D.L. and T.L. Wu. 1982. Atrazine Toxicity to Submersed Vascular Plants in Simulated Estuarine Microcosms. Aquatic Botany 14:151-158.

4. Correll, D.L., J.W. Pierce, and T.L. Wu. 1978. Herbicides and Submerged Plants in Chesapeake Bay. IN: Amer. Soc. Civil Eng. (ed.) Coastal Zone. 78 pp. 858-877.

5. Dao, T.H., T.L. Lavy, and R.C. Sorensen. 1979. Atrazine Degradation and Residue Distribution in Soil. Soil Sci. Soc. Am. J. 43:1129-1134.

6. Ebert, E., and S.W. Dumford. 1976. Effects of Triazine Herbicides on the Physiology of Plants. Residue Rev. 65:1-103.

7. Forney, D.R., and D.E. Davis. 1981. Effects of low concentrations of herbicides on submerged aquatic vegetation. Weed Sci. 29:677-685.

8. Goswami, K.P., and R.E. Green. 1971. Microbial degradation of the herbicide atrazine and its 2-hydroxy analog in submerged soils. Environ. Sci. Tech. 5:426-429.

9. Gysin, H., and E. Knusli. 1960. Chemistry and Herbicidal Properties of Triazine Derivatives. Adv. Pest Control Res. 3:289-358.

10. Jones, T.W., W.M. Kemp, J.C. Stevenson, and J.C. Means. 1982. Degradation of atrazine in estuarine water/sediment systems and soils. J. Environ. Qual. 11:632-638.

11. Jones, T.W., W.M. Kemp, P.S. Estes, J.C. Stevenson. 1982. Atrazine uptake phytotoxicity, release, and short-term recovery for the submerged aquatic plant Potamogeton perfoliatus. Report to U.S. E.P.A., Annapolis, Maryland, Grant No. R805932-01-1.

12. Kaufman, D.D., and J. Blake. 1970. Degradation of atrazine by soil fungi. Soil Biol. Biochem. 2:73-80.

13. Kemp, W.M., J.J. Cunningham, J.C. Stevenson, J.C. Means, W.R. Boynton. 1982. Response of submerged vascular plants to herbicide stress in estuarine microcosms. Report to U.S. E.P.A., Annapolis, Maryland, Grant No. R805932-01-1.

14. Pfister, K., K.E. Steinback, G. Gardner, and C.J. Arntzen. 1980. Identification of the photosystem II herbicide binding protein. Plant Physiol. 65(6):Supplement, Abstr. No. 48.

15. Pfister, K., S.R. Radosevich, and C.J. Arntzen. 1979. Modification of Herbicide Binding to Photosystem II in Two Biotypes of *Senecio vulgaris* L. Plant Physio. 64:995-999.

16. Shimabukuro, R.H. 1967. Significance of atrazine dealkylation in root and shoot of pea plants. J. Agri. Food Chem. 15:557-562.

17. Shimabukuro, R.H., and H.R. Swanson. 1969. Atrazine metabolism, selectivity, and mode of action. J. Agr. Food Chem. 17:199-205.

18. Sirons, G.R., R. Frank, and T. Sawyer. 1973. Residues of atrazine cyanazine and their phytotoxic metabolites in a clay loam soil. J. Agric. Food Chem. 21:1016-1020.

19. Skipper, H.D., and V.V. Volk. 1972. Biological and chemical degradation of atrazine in soils. Weed Sci. 20:344-347.

20. Stevenson, J.C., and N.M. Confer. 1978. Summary of available information on Chesapeake Bay submerged vegetation. Fish and Wildlife Service Pub. no. OBS-78/66, U.S. Dep. of the Interior. U.S. Government Printing Office, Washington, D.C.

21. Wolf, D.C., and J.P. Martin. 1975. Microbial decomposition of ring-^{14}C atrazine, cyanuric acid, and 2-chloro-4,6-diamino-s-triazine. J. Environ. Qual. 4:134-139.

22. Wu, T.L. 1980. Dissipation of the herbicides atrazine and alachlor in a Maryland corn field. J. Environ. Qual. 9:459-465.

CHAPTER VIII

EFFECTS OF NUTRIENT ENRICHMENT IN EXPERIMENTAL ESTUARINE PONDS CONTAINING SUBMERGED VASCULAR PLANT COMMUNITIES*

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TABLE OF CONTENTS

Page

LIST OF FIGURES.. VIII-iv
INTRODUCTION... VIII-1
MATERIALS AND METHODS.. VIII-2

- Ponds Design... VIII-2
- Experimental Design.. VIII-2
- Biomass.. VIII-4
  - Submerged Vascular Plants.................................. VIII-4
  - Epiphytic Material... VIII-4
  - Planktonic Material.. VIII-5
- Productivity and Respiration................................... VIII-5
  - Vascular Plants and Epiphytes.............................. VIII-5
  - Community Metabolism....................................... VIII-6
- Nutrient Dynamics.. VIII-6
  - Denitrification.. VIII-6
  - Nutrient Sampling.. VIII-6
  - Chemical and Physical Analyses............................. VIII-7
- Light Measurements... VIII-7

RESULTS.. VIII-8

- Nutrient Cycling... VIII-8
  - Water Column Concentrations................................ VIII-8
  - Plant Composition.. VIII-8
  - Sediment Nutrients... VIII-13
  - Denitrification Rates...................................... VIII-13
- Light Attenuation.. VIII-17
  - Epiphytic Solids... VIII-17
  - Water Column Attenuation................................... VIII-17
- Plant Biomass.. VIII-17
  - Submerged Vascular Plants.................................. VIII-17
  - Epiphytic Biomass.. VIII-21
- Plant and Epiphyte Productivity................................ VIII-24
  - Plant Production and Respiration........................... VIII-24
  - Plant/Epiphyte Relations................................... VIII-28
- Community Photosynthesis and Respiration....................... VIII-30

DISCUSSION... VIII-30

- Nutrient Uptake from the Water Column.......................... VIII-30
- Nitrogen Uptake and Storage by SAV............................. VIII-34
- Sediment Nutrient Dynamics..................................... VIII-39

Algal Response to Nutrient Enrichment........................ VIII-39
PAR Attenuation by Algal Biomass........................ VIII-42
Epiphyte Effects on SAV........................ VIII-45
SAV Biomass Response to Nutrient Enrichment........................ VIII-48
Response of Community Metabolism to Fertilization........................ VIII-50
SAV Strategy for Growth and Production........................ VIII-50

SUMMARY........................ VIII-52
LITERATURE CITED........................ VIII-54
APPENDICES........................ VIII-61

LIST OF FIGURES

Page

Figure 1. Diagram of pond mesocosms at Horn Point Environmental Laboratories. Broken lines represent the flow of estuarine water to and from the Choptank River estuary.. VIII-3

Figure 2. Time series plot of inorganic nitrogen (NH_4^+ and NO_3^-) and phosphorus concentrations (μM) in the pond water at four nutrient treatments beginning on 16 July, 1981................................. VIII-9

Figure 3. Concentrations (μM) of total nitrogen, nitrate, and ammonium in pond water at four nutrient treatments on the third day following nutrient applications during the summer, 1981................................. VIII-10

Figure 4. Tissue nitrogen (N) concentrations (% dry wt.) of Potamogeton perfoliatus subjected to four nutrient treatments at 26 and 60 days following the initial treatment.. VIII-11

Figure 5. Changes in the carbon:nitrogen ratios (atom weight) in tissues of Potamogeton perfoliatus subjected to four nutrient treatments during the summer, 1981......... VIII-12

Figure 6. Concentrations (μM) of nitrate plus nitrite and ammonium in the pore waters of sediments with and without vegetation in a low and high nutreint treated pond on 15 July 1981................................. VIII-14

Figure 7. Concentrations (μM) of nitrous oxide (N_2O) in the headspace at various intervals of a 24 h incubation of sediment cores froma low and high nutrient treatment. Also included are nitrate (NO_3^-) concentrations in water overlying the sediment cores at the beginning and end of the experiment..................... VIII-15

Figure 8. Denitrification rates ($\mu mol \cdot m^{-2} \cdot h^{-1}$) in the vegetated and bare sediments of a low and high nutrient treated pond..................................... VIII-16

Figure 9. Attenuation coefficients (m^{-1}) of the water column at four nutrient treatments........................ VIII-18

Figure 10. Concentrations of chlorophyll *a* ($\mu g/\ell$) and total seston (mg/ℓ) in the pond waters at four nutrient treatments.. VIII-19

Figure 11. Aboveground biomass (gdw/m^2) of submersed macrophytes in ponds subjected to four nutrient treatments. Fertilization began on 18 June, 1981........... VIII-20

Figure 12. a) Changes in the total biomass of submersed macrophytes (kg dry wt.) in the ponds subjected to four nutrient treatments. b) Average daily changes in the biomass of submersed macrophytes ($gdw \cdot m^{-2} \cdot d^{-1}$) in ponds at four nutrient treatments........................ VIII-22

Figure 13. Epiphyte biomass (gdw/gdw(SAV)) on *Ruppia maritima* and *Potamogeton perfoliatus* in ponds subjected to four nutrient treatments. Fertilization began after the June measurements.. VIII-23

Figure 14. Epiphyte biomass (chl-*a*/gdw (SAV) on *Ruppia maritima* and *Potamogeton perfoliatus* in ponds subjected to four nutrient treatments. Fertilization began after the June measurements.. VIII-25

Figure 15. Photosynthetic active radiation ($\mu Ein \cdot m^{-2} \cdot s^{-1}$) at the water's surface and at 0.5 m depth from midmorning to midafternoon along with measurements of apparent production (P_a) and net respiration (R_n) for *Ruppia maritima* and *Potamogeton perfoliatus*................ VIII-26

Figure 16. Net photosynthesis ($mg\ C \cdot gdw^{-1} \cdot h^{-1}$) along the length of *Potamogeton perfoliatus* and *Ruppia maritima* cleared of epiphytic material. Included is the amount of photosynthetic active radiation ($\mu Ein \cdot m^{-2} \cdot s^{-1}$) from water's surface to 0.5 m depth.. VIII-27

Figure 17. Apparent production and net respiration [mg O_2 (g dry wt. SAV)$^{-1} \cdot h^{-1}$] of *Potamogeton perfoliatus* and associated epiphytes from a low nutrient and a high nutrient treated pond.. VIII-29

Figure 18. Net photosynthesis [mg C (g dry wt. SAV)$^{-1} \cdot h^{-1}$] of the tip (0-15 cm) and basal (15-50 cm) sections of *Potamogeton perfoliatus* and *Ruppia maritima* and associated epiphytes.. VIII-31

Figure 19. Photosynthesis and respiration (g $O_c \cdot m^{-3} \cdot d^{-1}$) of the pond communities at four nutrient levels during summer of 1981.. VIII-32

Figure 20. Changes in net production ($g\ O \cdot m^{-3} \cdot d^{-1}$) of the pond communities at four nutrient treatments during the summer of 1981. Fertilization began after the June measurements.................................... VIII-33

Figure 21. Uptake rate for NH_4^+ and NL_3^- from pond waters with submersed macrophyte communities at various concentrations of nitrogen.. VIII-35

Figure 22. The relationship between tissue nitrogen content (%) and biomass production of submersed macrophytes ($gdw \cdot m^{-2} \cdot d^{-1}$).. VIII-37

Figure 23. The response of photosynthetic active radiation (attenuation coefficient, K), plankton chlorophyll *a* (µm/ℓ) and total sestion (mg/ℓ) of the water in pond mesocosms subjected to four nutrient treatments.. VIII-41

Figure 24. Correlations among the attenuation of photosynthetic active radiation of the water column in ponds treated with nutrients and A) total seston concentration (mg/ℓ), and B) total nitrogen concentration (µm)............ VIII-43

Figure 25. Correlations between A) attenuation coefficients for photosynthetic active radiation and plankton chlorophyll *a*, and B) total nitrogen concentrations (µm) and plankton chlorophyll *a* in water column of ponds treated with nutrients............................. VIII-44

Figure 26. Effects of epiphyte biomass [gdw/gdw(SAV)] on the normal apparent production of *Potamogeton perfoliatus* (% of production of plants cleared of epiphytic material) based on experiments using ^{14}C-incorporation and dissolved oxygen evolution to measure plant metabolism.................................... VIII-46

Figure 27. Effects of epiphyte biomass on *Potamogeton perfoliatus* expressed as area (mg/cm^2) and weight [gdw/gdw(SAV)].. VIII-47

Figure 28. The response of submersed aquatic vegetation in experimental ponds to various loading rates of nitrogen and phosphorus.............................. VIII-49

Figure 29. The apparent production to nighttime respiration ratio of ponds at four nutrient treatments........................ VIII-51

INTRODUCTION

Over the past two decades there has been a marked decline in abundance and distribution of submerged vascular plants in Chesapeake Bay (Bayley et al. 1978; Stevenson and Confer 1978; Orth and Moore 1981). While densities of *Zostera marina* have been reduced in the higher salinity reaches of this estuary, the most dramatic losses have occurred in the upper and middle bay. Here, numerous plant species have been involved, such as *Potamogeton perfoliatus*, *P. pectinatus*, *Ruppia maritima*, *Zannichellia palustra*, *Elodea canadensis* and *Valisneria americana*. A number of factors have been suggested as contributing to this decline, including the increasing input of nutrient wastes (Boynton et al. 1981). Nutrient enrichment is well documented for several regions of Chesapeake Bay, as is the increase in phytoplankton chlorophyll *a* (Mihursky and Boynton 1979; Heinle et al. 1981). Prior to the general demise of submerged aquatic vegetation (SAV) in the Bay, there was a proliferation of the exotic species *Myriophyllum spicatum* throughout the upper bay (Bayley et al. 1978). This is similar to the pattern reported by Lind and Cottam (1969), where *Myriophyllum* invasion of Lake Mendota occurred in response to eutrophication.

Various investigators have postulated that observed losses of SAV in other freshwater systems were attributable to eutrophication, including Loch Leven, Scotland (Jupp and Spence 1976), Lake Erie, Ohio (Stuckey 1978), Whitewater Lake, Ontario (Dale and Miller 1978), and Norfolk Broads, England (Phillips et al. 1978). Others have suggested that nutrient enrichment may be one of several factors contributing to dimunition of seagrasses in such coastal ecosystems, as the Dutch Waddenzee (Den Hartog and Polderman 1975), the French Mediterranian (Peres and Picard 1975), and Cockburn Sound, Australia (Cambridge 1979). The hypothesized mechanisms whereby nutrient additions could lead to reductions in SAV involve the promotion of algal growth, either phytoplanktonic (Jupp and Spence 1977) or epiphytic (Phillips et al. 1978), and a resulting reduction in light available to the vascular plants (Sand-Jensen 1977; Penhale 1977). Additional stress mechanisms may be involved such as: inhibition of molecular transport across SAV epidermis (Sand-Jensen 1977); enhanced deposition of sediments on SAV leaves (Menzie 1979); and incresed growth of colonial animal fouling on SAV leaves stimulated by elevated availability of phytoplankton (Staver et al. 1980).

Much of the information regarding nutrient effects on algal-SAV relationships has been inferred from indirect correlative evidence along natural nutrient gradient (e.g. Cattaneo and Kalff 1980; Sand-Jensen and Søndergaard 1981). However, direct experimental approaches have been applied for freshwater ecosystems utilizing pond or pool microcosms (Mulligan and Baranowski 1969; Ryan et al. 1972; Moss 1976; Mulligan et al. 1976; Phillips et al. 1978). In fact, early experiments with fish ponds demonstrated that fertilization offered an effective means of controlling aquatic weeds (e.g., Smith and Swingle 1941). In a recent paper Harlin and Thorne-Miller (1981) reported the results of semicontrolled experiments where nutrients were added to seagrass beds in situ. While this study provided some provocative results, rates of nutrient additon could be determined only qualitatively. With this exception, there have been no direct experiments investigating nutrient enrichment effects on SAV communities for marine or estuarine systems.

In this paper we report the results of a study where we used eight replicate experimental estuarine ponds stocked with mixed SAV species and flushed with Chesapeake Bay waters. The purpose of this study was to investigate the effects of nutrient enrichment on: (1) abundance of phytoplankton and epiflora; (2) community production and nutrient cycling; (3) interactions between algae and SAV (especially relative to light availability); and (4) SAV growth, abundance and nutrient status.

MATERIALS AND METHODS

Ponds Design

The eight rectangular ponds were constructed at Horn Point Environmental Laboratories during the spring and summer of 1979 (Fig. 1). The dimensions of each pond are 27 m by 13 m at water surface, with sides sloped (1:1) to a nominal depth of about 1.2 m. Water from the Choptank River is pumped to a centrally located reservoir, from which water flows into each pond. Estuarine water entered each pond through a control valve and exited through a standpipe, so that flushing rate and water level could be controlled. The ponds have three piers each to facilitate sampling. Two short (3 m) piers are located at the inflow and outflow ends of the ponds, and a longer pier is located midway along one side, extending 9 m into the pond.

The ponds were stocked with submersed aquatic vegetation during the summer of 1979 from coves located along the Choptank River estuary. Cores (15 cm dia) containing plant shoots, roots and sediments were transported from source areas to the ponds, where they were transplanted in a grid with 1 m distance between adjacent plugs. Only two diagonally separated quarters of each pond were seeded. The dominant transplanted SAV species were Potamogeton perfoliatus and Ruppia maritima. By the summer of 1980, about 50-80% of the sediment surface was covered by SAV, and several other vascular plant species were observed at lower densities, including Zannichellia palustrus, Myriophyllum spicatum, and Elodea canadensis. This experiment was initiated in the early summer of 1981, and represented the first manipulative treatment of these transplanted grass beds.

Experimental Design

Three different treatments of dissolved nutrients were applied to duplicate ponds, and two ponds served as controls. Commercial fertilizers in the form of ammonium sulphate, potassium nitrate, and di-ammonium phosphate were dissolved in distilled water and applied to the ponds with a sprayer. The nitrogen (N) application rates were 1.68, 0.84, and 0.42 g/m^2 for the high, medium, and

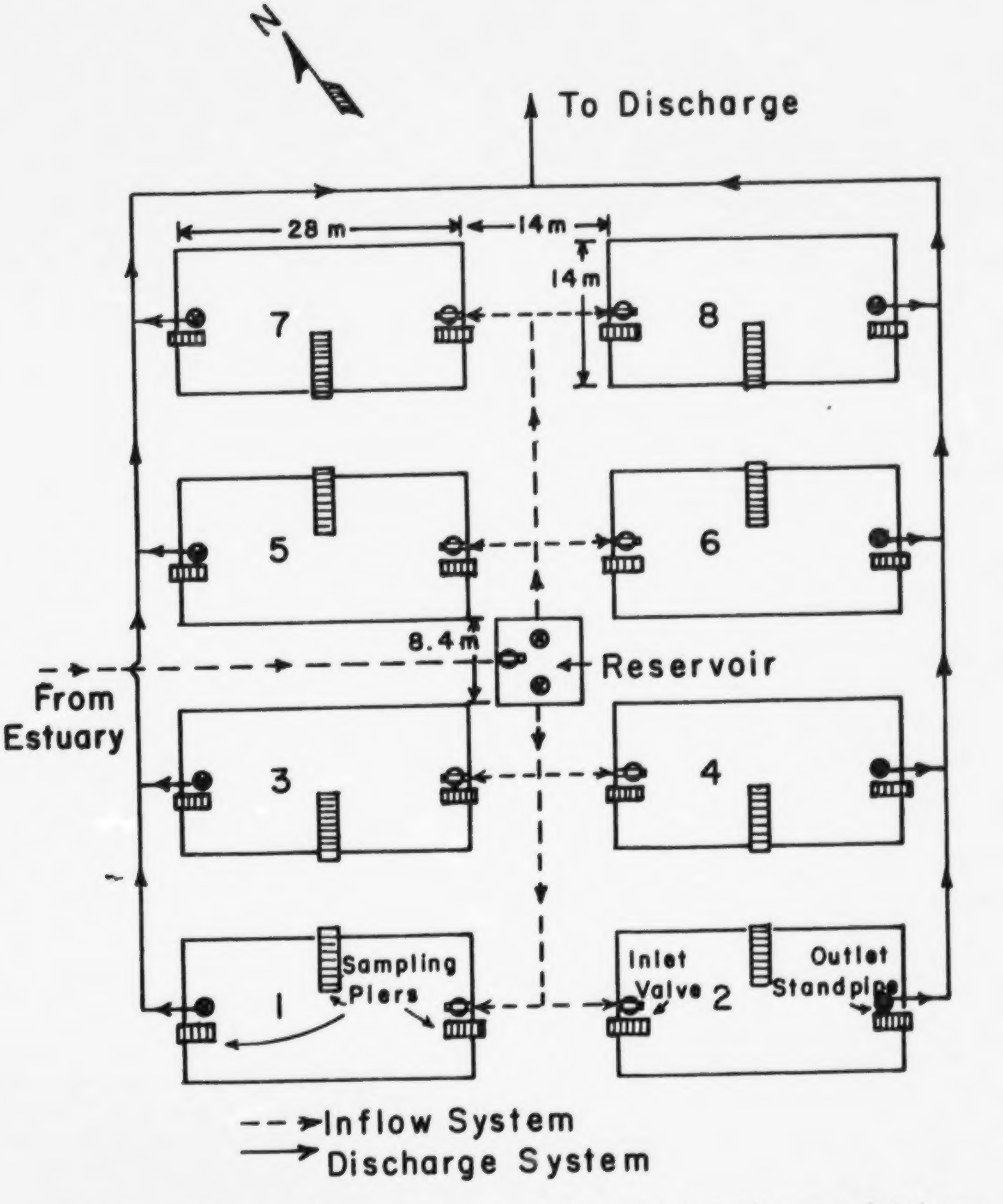
N
To Discharge
28 m
14 m
14 m
7
8
5
6
8.4 m
Reservoir
From Estuary
3
4
1
2
Sampling Piers
Inlet Valve
Outlet Standpipe
Inflow System
Discharge System

Figure 1. Diagram of pond mesocosms at Horn Point Environmental Laboratories. Broken lines represent the flow of estuarine water to and from theChoptank River estuary.

low dose ponds, respectively, and contained equal amounts of nitrate (NO_3^-) and ammonium (NH_4^+). Concentrations of inorganic N immediately after dosing were 120, 60, and 30 μM in treated ponds, respectively. Corresponding phosphorus (P) loading rates were 0.37, 0.19, and 0.09 g/m^2 which yielded initial concentrations of 12, 6 and 3 μM in treated ponds. The ponds were treated on six occasions at 10 d intervals beginning 19 June 1981 and ending on 4 August 1981. The ponds were held in batch mode for 8-9 d following each application, followed by 1-2 d of flushing with ambient estuarine water to effect a complete turnover of water.

Biomass

Submerged Vascular Plants

Submersed aquatic vegetation was sampled in each pond twice before fertilization began; twice during the treatment period and once during post treatment in November. A stratified random sampling procedure was used to select six 0.1 m^2 vegetated plots in each pond. Aboveground (leaves and stems) and belowground (roots and rhizomes) biomass was harvested on 17 June whereas only shoots were sampled on the other occasions. Samples were rinsed with estuarine water, sorted by species, and dried in a forced draft oven at 60°C to constant weight. Dry weights were determined to the nearest 0.01 g. The dried biomass was ground through a 40 mesh screen using a Wiley Mill and stored in air tight plastic bags. Aerial photography at 150 m was used to determine the percent cover of SAV in each pond on each sampling date. Kodak Ektachrome ASA 400 daylight film was used to produce color slides from which the cover of SAV was traced and area determined.

The chlorophyll content of *Potamogeton* was determined from 3 mm diameter disks sampled from leaves along the terminal 10 cm section of the plant. These samples were ground in a 10:1 dimethylsulfoxide (DMSO): acetone solution (Hiscox and Israelstam 1979) and chlorophyll absorption was measured on a Cary 219 spectrophotometer.

Epiphytic Material

Epiphyte biomass was determined each time SAV biomass was measured except for the first pretreatment and the post-treatment occasions. Individual plants were sampled in triplicate and placed in 1 ℓ plastic bottles. Epiphytes were removed by agitating the bottles for several minutes. This water sample was stirred in a beaker while three ten ml aliquots were removed and filtered through preweighed GFC glass fiber filters. These three subsamples were duplicated for analysis of chlorophyll *a* and total suspended solids. Chlorophyll *a* filters were wrapped in aluminum foil and frozen, and the others were dried for two days at 60°C. Background total suspended solid concentrations were determined on pond water alone. The plant from which epiphytes were removed was dried in a plant press. After determining dry weights, these samples were analyzed for leaf area with a LICOR Model 3100 area meter.

Planktonic Material

Plankton pigments were measured by filtering 100-200 ml of pond water through 0.45 μ GFC filters. Chlorophyll-a was extracted for 24 h in 10 ml of a 1:1 solution of DMSO and acetone (Shoaf and Lium 1976) and flourescence was measured using a Turner Model 111 flourometer.

Productivity and Respiration

Vascular Plants and Epiphytes

Primary production and respiration were measured using both dissolved oxygen (DO) and ^{14}C techniques. Total seston was determined again by filtration of 50-200 ml of ambient water through GFC filters (0.45 μ). Filters were washed, dried and weighed according to the methods of Strickland and Parsons (1972). SAV and/or epiphytes were incubated in 300 ml biological oxygen demand (BOD) bottles filled with filtered (1.0 μ) estuarine water, and changes in DO over time were used to estimate apparent productivity. Bottles were incubated for short time intervals (1-3 h) in either the ponds or in a 0.25 m deep incubator under ambient light and temperature with continuously circulated river water. Light attenuation, salinity, and temperature of the water column were also measured during each experiment.

The productivity of the top 15 cm of plants with epiphytes of various densities were compared to the basal sections of SAV with epiphytes. The contribution of epiphytes to DO changes within these bottles was calculated by extrapolating measured rates of just epiphyte production to results with epiphytes plus SAV. The epiphyte biomass on the plants (g/gdw(SAV)) was measured as discussed above and used to convert epiphyte production data (mgO_2 gdw $(epiphyte)^{-1}$) to epiphyte production associated with SAV. DO changes in light bottles represented apparent net production and no corrections were made for oxygen storage since lacunal capacity in these plants is minor (Westlake 1978). DO changes in opaque bottle incubations were used to provide an estimate of dark respiration.

Net carbon incorporation was also measured using uptake of ^{14}C bicarbonate. Whole plants were carefully removed from sediments and placed with epiphytes intact into clear plexiglass tubes (7.5 cm dia, 50 cm long) with the roots of the plants attached to one end of the core. These cores were sealed, and floated vertically in the control pond such that the tip of the shoots were 2-3 cm below water's surface and the remainder of the plant extended to 0.5 m depth (the length of the core). Carrierfree ^{14}C-bicarbonate was injected along the length of the core via to a specific activity of 5 μCi/ℓ. Plants were incubated for 1-2 h during midday.

Potamogeton perfoliatus was studied at 2 epiphyte levels and *Ruppia maritima* at only 1 epiphyte level, and controls for both plants were nearly free of epiphytes. Following incubation, the control plants were sectioned into 10 cm lengths, and the epiphyte colonized plants were sectioned into two lengths which were either less than or greater than 15 cm from the tip of the plant. The epiphytes were removed with a stream of water and a rubber policeman, and the entire contents filtered onto GFC glass fiber filters. Plants and filters were dried to constant weight at 60°C and digested in nitric acid according to Lewis et al. (1982). Radioassay was performed with a Packard Tri-Carb 460C liquid scintillation counter standardized with a quench curve. The absolute amount of CO_2 uptake was determined from specific activity and alkalinity measurements, which represents net productivity.

Community Metabolism

Diel changes of DO in the ponds were used to estimate community production and respiration following the approach of Odum and Hoskin (1958). Measurements were made prior to and during fertilization. DO and temperature measurements were made at the surface and 0.5 m at 3 stations (the end of each sampling pier) in each pond at dawn and at dusk. This sampling scheme was performed for 2 consecutive days 7 times during the summer. Changes in DO were corrected for oxygen transfer across the air-water interface using a modification of the method of Copeland and Duffer (1967). Changes in DO from dawn to dusk and from dusk to dawn were taken to represent net production and nighttime respiration, respectively.

Nutrient Dynamics

Denitrification

Duplicate intact sediment cores (7.5 cm diameter x 20 cm depth) were obtained in vegetated and unvegetated areas of a low nutrient and high nutrient dosed pond. Sediment denitrification was measured in each core following the acetylene reduction procedure of Sorensen (1978). The overlying water in each core was removed to give a headspace of 4 cm resulting in a water depth of about 15 cm. Cores from the low and high nutrient dosed ponds, were enriched with dissolved nitrate at concentrations of 15 μM N and 60 μM N above ambient levels, respectively. The cores were sealed and the headspace purged with a gas mixture (80% Ar, 18% O_2, 2% CO_2) to remove nitrogen while providing an *in situ* oxygen tension. Acetylene (C_2H_2) was injected into the core to achieve a partial pressure of 0.2 atm (Patriquin and Denike 1978), and the headspace of the core was sampled at 0, 3, 6, 17 and 24 h for N_2O evolution.

At 24 h the headspace was again purged with the gas mixture, amended with acetylene and incubated for another 24 h period. At 0, 24 and 48 h the overlying water was sampled for dissolved inorganic nutrients (NH_4^+, NO_3^- and DIP) and DO. Cores were incubated in Percival incubators at 30°C and a 16 h light, 8 h dark photoperiod. Headspace samples were stored in vacutainers and later analyzed by a Packard 111 gas chromatograph equipped with a ^{63}Ni electron capture detector. Standards were run using pure nitrous oxide (Air Products, Inc.) to relate peak heights to N_2O concentration.

Nutrient Sampling

Water. Water in each pond was sampled at a depth of about 0.5 m at the 3 stations (end of each pier) in each pond throughout the summer. Unfiltered subsamples were stored in plastic bottles and frozen for subsequent analysis of total N and P. Triplicate 150 ml aliquots were filtered through preweighed GFC glass fiber filter paper (1 μ) immediately following collection for analysis of both chlorophyll a and total suspended solids. Filters for chlorophyll a were wrapped in aluminum foil and frozen, while the other filters were dried for several days at 60°C and weighed. The filtered water samples were analyzed for NH_4^+, NO_3^-, NO_2^- and DIP. Total nitrogen and phosphorus, and pH were measured on unfiltered samples.

Sediment. During the 17 June and 13 August biomass samplings, 6 sediment samples were taken in each pond with a modified plastic core (2.5 cm dia, 15 cm long). These sediment cores were sectioned at 2 cm intervals to a depth of 10 cm. Fresh weights were measured, the samples were dried to constant weight at 60°C and weighed again to obtain water content. Each section was ground with a mortar and pestle, stored in plastic-pak bags and analyzed for total carbon, nitrogen and phosphorus. Four intact sediment cores (7.5 cm diameter x 30 cm length) were taken in a low and high nutrient dosed pond on 15 July 1982. Two cores in each pond were from vegetated and unvegetated areas. Each core was sectioned into 2 cm subsamples to a depth of 10 cm in a glove-box under N_2 atmosphere. Triplicate subsamples from each section were combined and extracted with deionized water. Following centrifugation, the supernatant was filtered through GFC filters and analyzed for inorganic nutrients (NH_4^+, NO_3^- plus NO_2^-, DIP).

Chemical and Physical Analyses

Inorganic nutrients were assayed on a Technicon Auto Analyzer II using the following techniques: ammonium determinations followed Solorzano (1969); nitrate was reduced with a cadmium column and nitrite was reacted with sulfanilamide and N-1-dihydrochlomide napthylene diamine; phosphorus results represent filterable phosphorus reactive with ammonium molybdate in ascorbic acid. Total phophorus and nitrogen were digested with persulphate using the method of Valderrama (1981) followed by inorganic phosphate and nitrate assay described above. pH was measured with a Beckman Model 4500 digital pH meter with a calomel reference electrode. An Orbisphere Model 2603 oxygen meter and probe (with stirrer) were used to measure DO in open water and in BOD bottles. Total carbon and nitrogen of sediment, plant and filter paper were assayed with a Perkin Elmer 240B elemental analyzer.

Light Measurements

Attenuation of photosynthetically active radiation (PAR) in each pond was determined periodically throughout the summer with a LICOR 185A light meter equipped with a LICOR 1925B underwater quantum sensor. Daily integral light energy and PAR were also obtained during all productivity experiments using a Weather Measure Corp. Model No. R401 pyronometer and the LICOR meter.

Light attenuation due to epiphytes was determined using a modification of the method of Borum and Wium-Andersen (1980). Here, the reduction in PAR was measured as it passed through a finger bowl containing different densities of epiphytes removed from Potamogeton perfoliatus leaves. Total suspended solid concentration of the epiphyte medium was used to determine the weight of epiphytes per cm^2 in the bowls. This value was related to percent of original light to calculate light attenuation coefficients.

RESULTS

Nutrient Cycling

Water Column Concentrations

During the week following each nutrient dosing, inorganic nutrient concentrations underwent a rapid and steady decline in all ponds (Fig. 2). Initial concentrations varied according to treatment, but within 3 d NH_4^+ and DIP concentrations in all ponds were dramatically reduced. The exception was NO_3^- in the high treatment ponds which remained above 25 μM even after 7 d, Whereas NO_3^- in the other ponds was less than 1.0 μM after 3 d.

Concentrations of NO_3^-, NH_4^+, and DIP, as well as total P and total N were measured throughout the summer on the third day following treatment (Appendix, Table A-1 to A-5). The trend apparent in Fig. 2, where NH_4^+ and DIP were reduced to similar levels among treatments within 3 d, was exhibited for each dosage except the last. Following the sixth dosage, both NH_4^+ and NO_3^- remained relatively elevated in the high dose ponds (Fig. 3). Although the inorganic nutrients were similar among treatments after 3 d, concentrations of total N and P reflected treatment levels throughout the summer (Fig. 3). Over 90% of the total nitrogen levels were organic N in the control, low and medium treatment ponds. Inorganic N typically comprised about 25% of the total for high treatment systems, except in the 6th wk when NH_4^+ and NO_3^- were nearly 50% of total N (Fig. 3).

Plant Composition

The N content in Potamogeton perfoliatus under control conditions changed little from mid-June to August (Fig. 4, Table B-1). However, nitrogen levels of plants in the low and medium nutrient dosed ponds increased gradually during the summer by about 0.3% N for each treatment. The most dramatic change in % N was observed in the SAV in the high nutrient treatment, where % N during the course of the experiment increased from 1.10-1.99% N in June to 3.07% N in August.

These changes in % N (g dry wt.) for Potamogeton are also reflected in the carbon to nitrogen atom ratios in plants among the treatments (Fig. 5, Table B-2). C:N ratios of plants increased in the control ponds from 16 to 19, yet the ratios decreased in the other treatments. The magnitude of this decrease in C:N ratios among the treatments followed the nutrient application rate, with the greatest decrease observed in SAV from the high nutrient treatment. C:N ratios of Potamogeton in these ponds were only 10:1 in August. These changes in C:N ratio and % N demonstrate the capacity of SAV to take up N luxuriously in response to increased nutrient loading.

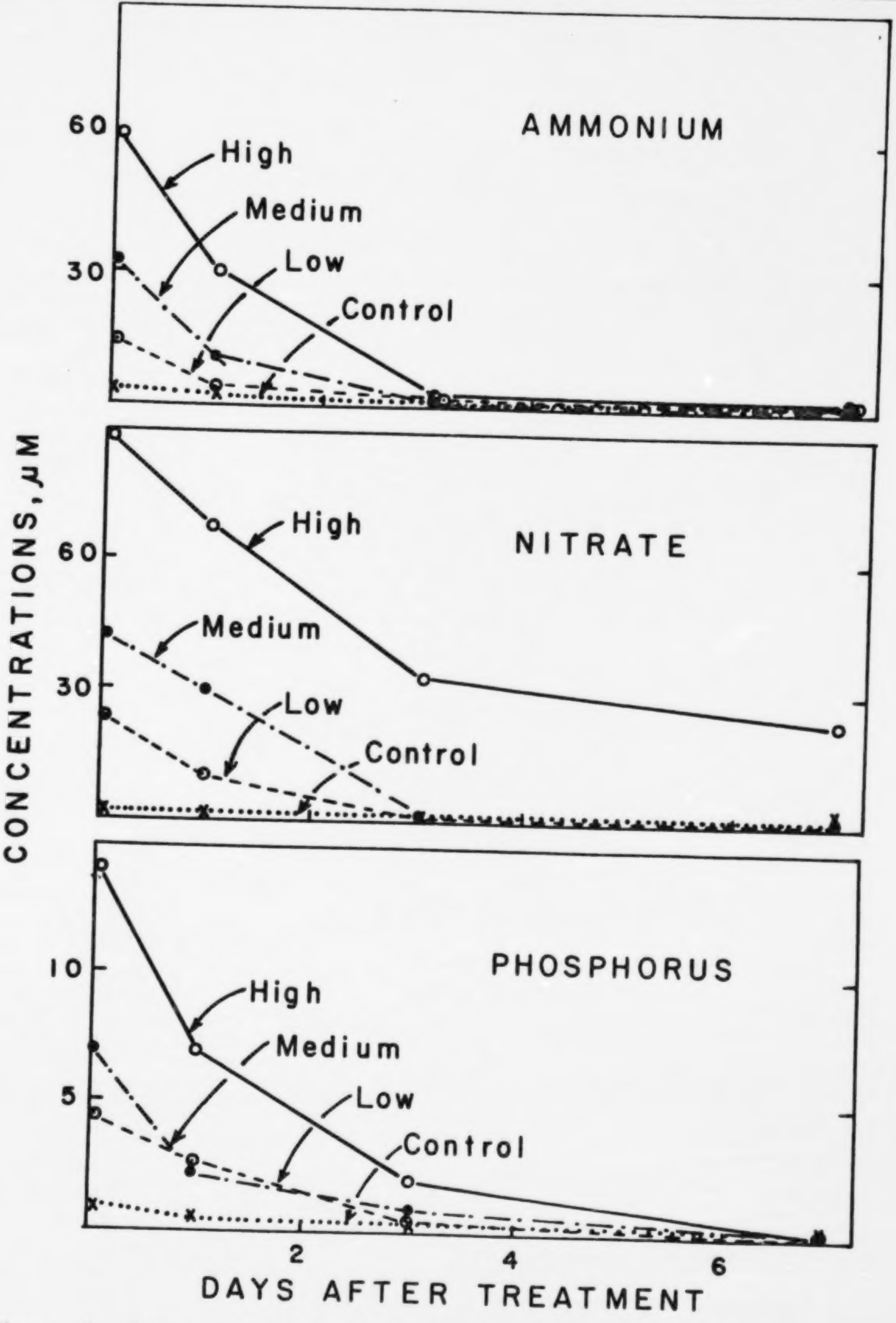

Figure 2. Time series plot of inorganic nitrogen (NH_4^+ and NO_3^-) and phosphorus concentrations (μM) in the pond water at four nutrient treatments beginning on 16 July, 1981.

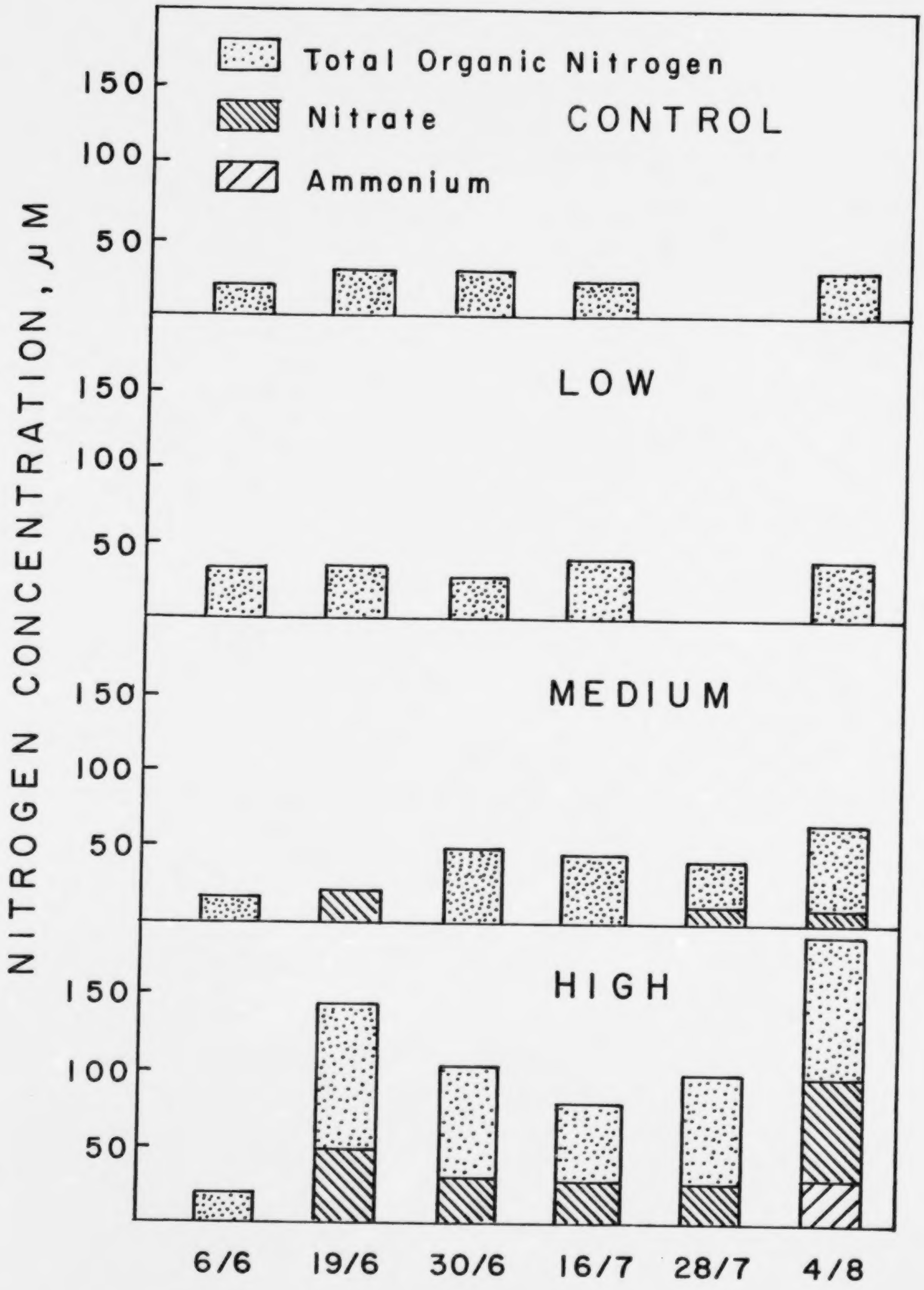

Figure 3. Concentrations (μM) of total nitrogen, nitrate, and ammonium in pond water at four nutrient treatments on the third day following nutrient applications during the summer, 1981.

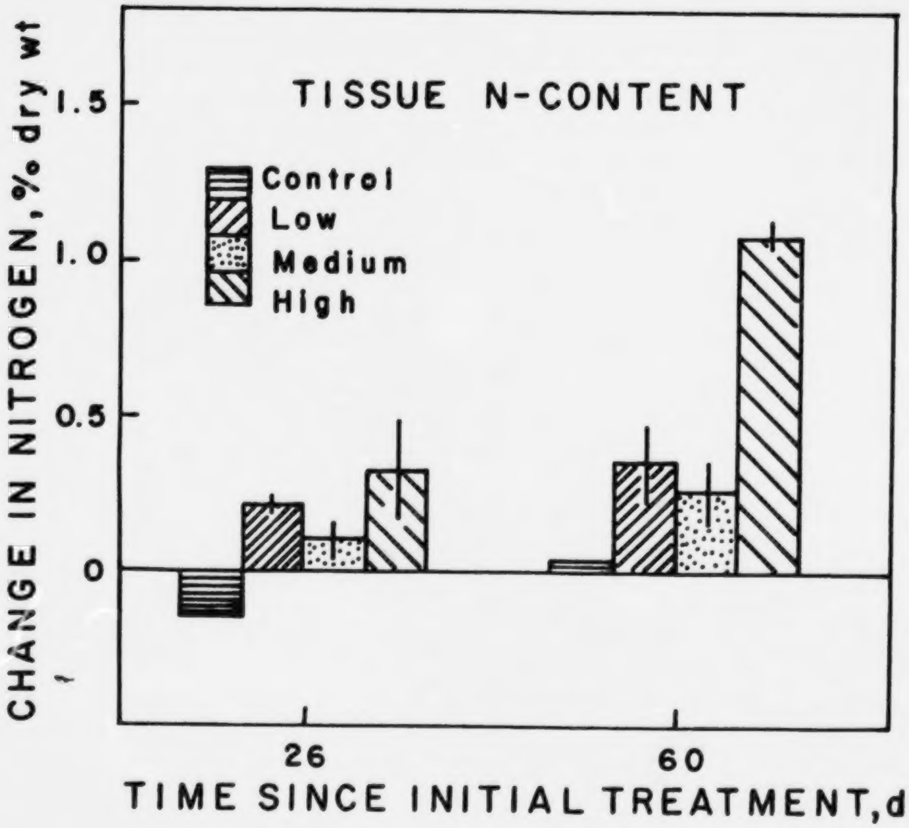

Figure 4. Tissue nitrogen (N) concentrations (% dry wt.) of Potamogeton perfoliatus subjected to four nutrient treatments at 26 and 60 days following the initial treatment.

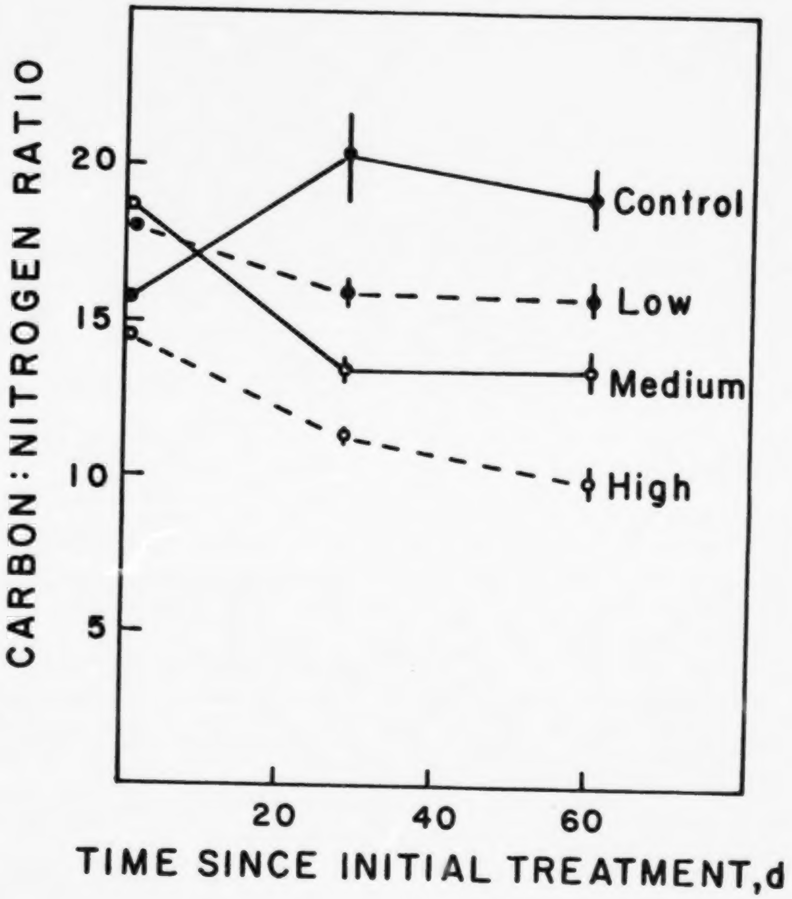

Figure 5. Changes in the carbon:nitrogen ratios (atom weight) in tissues of Potamogeton perfoliatus subjected to four nutrient treatments during the summer, 1981.

Sediment Nutrients

Nitrate concentrations in the interstitial pore waters of sediments were similar between the low and high nutrient dosed ponds in July (Fig. 6). Neither were there any distinct trend in NO_3^- of the pore waters in vegetated compared to unvegetated areas in either pond. In contrast, NH_4^+ concentrations of the pore waters in the high nutrient dosed pond were consistently higher than those in the low nutrient pond (Fig. 6). Concentrations ranged from 150 μM to 400 μM in the former pond compared to a range of 40 μM to 210 μM in the lower nutrient treatment.

Ammonium concentrations were about 180 μM in the vegetated and unvegetated areas of the high nutrient pond in the upper 6 cm. From 6 to 10 cm sediment depth, NH_4^+ in the unvegetated areas increased sharply to about 350 - 400 μM, whereas NH_4^+ levels in the vegetated cores remained at concentrations less than 200 μM. In the low nutrient dosed pond, pore-water NH_4^+ in the vegetated areas was usually only 25% of NH_4^+ concentrations in unvegetated areas to a depth of 10 cm. Concentrations of the vegetated areas did not exceed 90 μM, whereas concentrations were generally 180 μM in the unvegetated areas (Fig. 6).

Denitrification Rates

Nitrous oxide (N_2O) production in the low NO_3^- dosed core (10 μM) varied considerably during the first 24 h incubation period (Fig. 7). N_2O evolution was low during the first 3 h of incubation reflecting the lag for acetylene to diffuse into the sediments. From 3 to 6 h a sharp pulse of N_2O was produced in all cores. Following this pulse, N_2O production rates declined, and differences were observed among cores with and without SAV. The change in N_2O production during the time course can be related to the depletion of NO_3^- concentrations during the experiment. The initial NO_3^- concentration was 10 μM in the low nutrient dosage which decreased to about 1.1 μM at 17 h. Since denitrification rates are dependent on NO_3^- concentrations, a reduction in N_2O production would be expected as NO_3^- concentrations in the overlying water decreased.

The time series of N_2O production in the high nutrient dosed cores also showed a lag in rates during the first 3 h (Fig. 7). N_2O production was generally linear from 3 to 17 h although some decrease in rates was observed from 17 to 24 h. The change in NO_3^- concentration was from 72 to 4 μM, and therefore lower N_2O production rates at 17 h could be expected because of the much lower NO_3^- levels in the water column.

Denitrification rates were calculated from changes in N_2O from 3 to 6 h for the vegetated and unvegetated cores of both nutrient levels (Fig. 8). The rates were significantly greater in high dosed cores, with values ranging from 19 to 22 $\mu mol \cdot m^{-2} \cdot h^{-1}$ in low dosed cores and from 44 to 46 $\mu mol \cdot m^{-2} \cdot h^{-1}$ under high nutrient dosage (Fig. 8). There were no significant differences between the vegetated and unvegetated cores for either nutrient treatment.

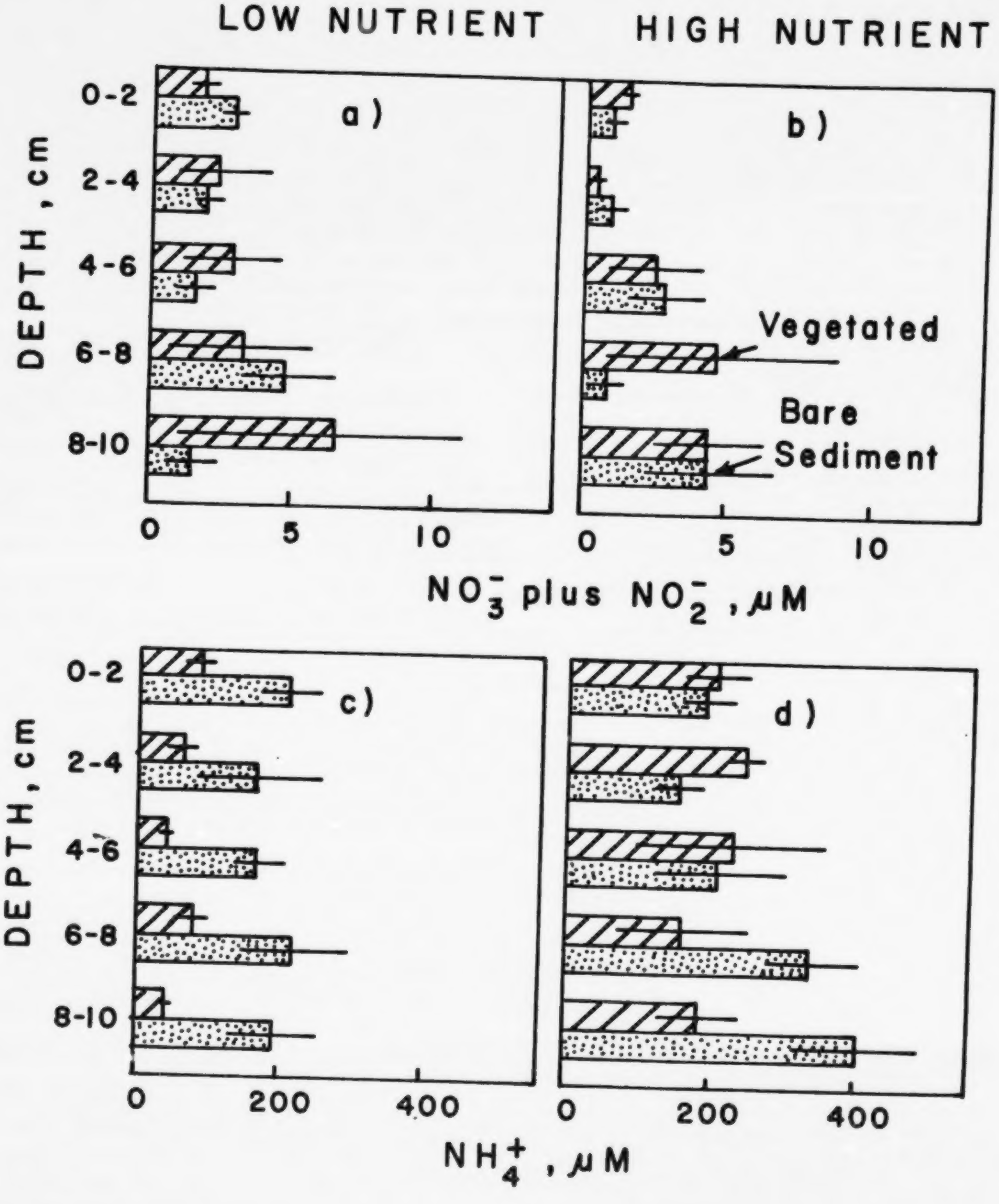

Figure 6. Concentrations (μM) of nitrate plus nitrite and ammonium in the pore waters of sediments with and without vegetation in a low and high nutrient treated pond on 15 July 1981.

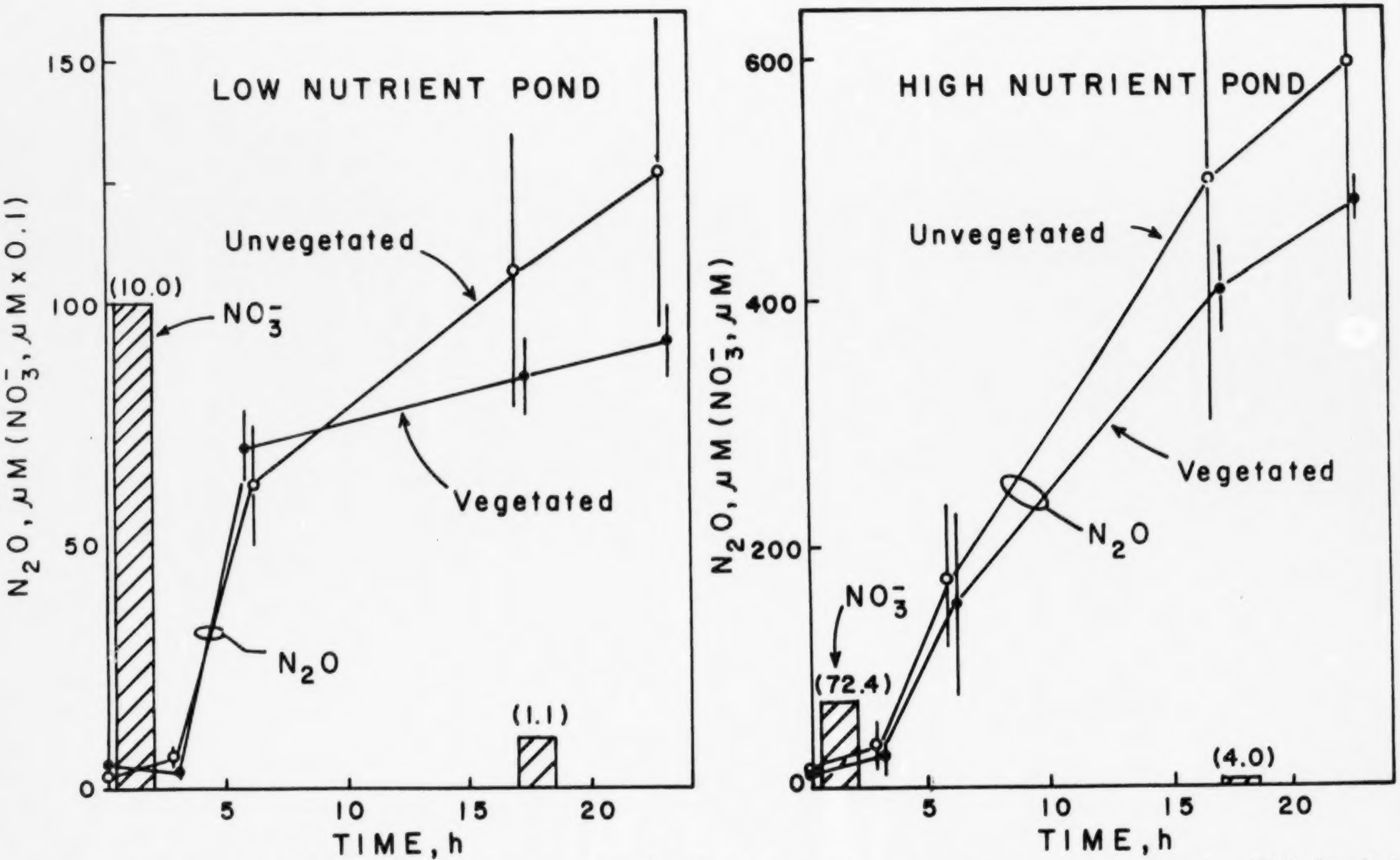

Figure 7. Concentrations (μM) of nitrous oxide (N_2O) in the headspace at various intervals of a 24 h incubation of sediment cores from a low and high nutrient treatment. Also included are nitrate (NO_3^-) concentrations in water overlying the sediment cores at the beginning and end of the experiment.

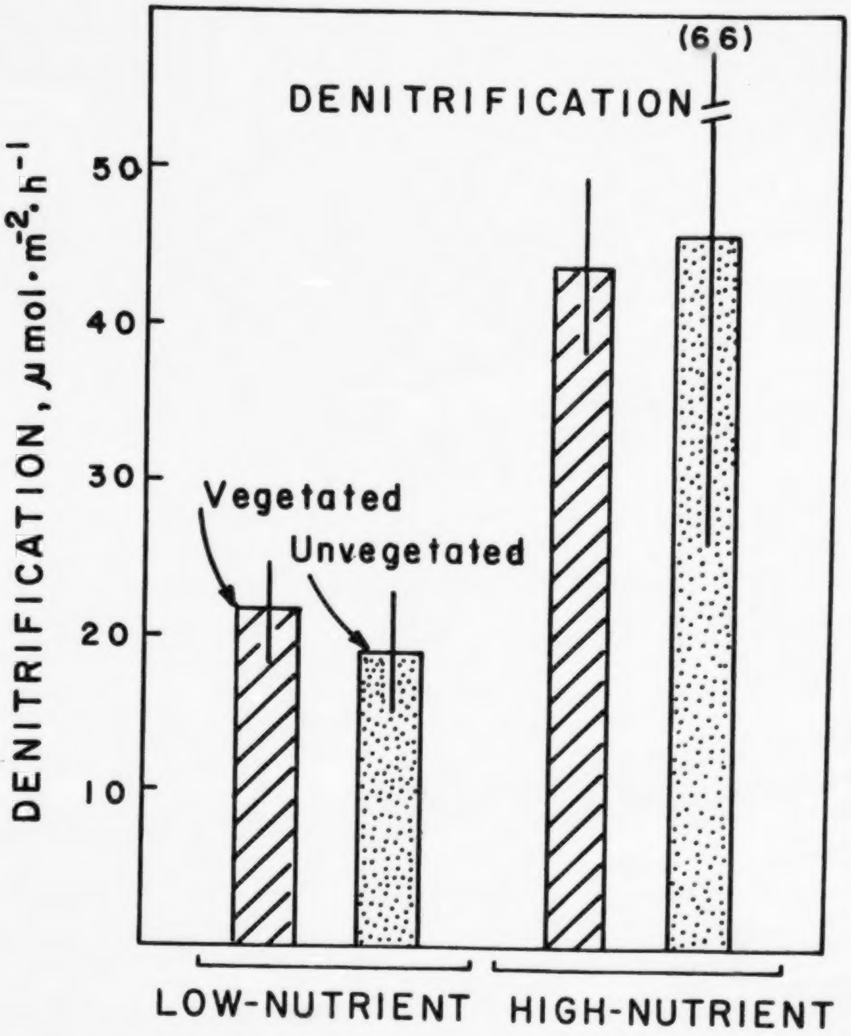

Figure 8. Denitrification rates ($\mu mol \cdot m^{-2} \cdot h^{-1}$) in the vegetated and bare sediments of a low and high nutrient treated pond.

Light Attenuation

Epiphytic Solids

The attenuation of PAR by epiphytic solids on the leaves of *Potamogeton* followed a negative exponential relation ($Y = Y_o e^{-0.36x}$, $r^2 = 0.87$). This estimate is based on the dry weight of total solids on the leaf surface. Presumably, the relationship between light attenuation and chlorophyll content of epiphytes might indicate a greater effect per unit pigment due to high absorption in the spectral range from 400 to 700 nm.

Water Column Attenuation

Attenuation of PAR by the water column in experimental ponds was not significantly different among control, low, and medium nutrient dosed systems, but was much greater under high nutrient treatment (Fig. 9). Attenuation coefficients (K) in the control ponds were about 1 m^{-1}, while values were slightly higher (1.0 to 1.5 m^{-1}) in low and medium nutrient treatments. Turbidity in high nutrient ponds was significantly greater overall, with PAR attenuation coefficients averaging about 4.0 m^{-1} and reaching 8.0 m^{-1} during the final sampling. This attenuation coefficient of 4 m^{-1} represented a reduction of typical ambient midday light (1200 $\mu E\ m^{-2} \cdot s^{-1}$) from 730 $\mu E\ m^{-2} \cdot s^{-1}$ just below the water surface to about 160 $\mu E\ m^{-1} \cdot s^{-1}$ at 0.5 m deep.

High nutrient ponds had elevated concentrations of total suspended solids and chlorophyll *a* compard to other treatments (Fig. 10). Whenever attenuation coefficients were greater than 3 m^{-1}, chlorophyll *a* concentrations exceeded 150 µg/ℓ. Chlorophyll *a* above 20 µg/ℓ was observed only under high dosage. However, while total suspended solid (TSS) levels were about 30 mg/ℓ for control and low nutrient treatments; they were significantly greater (ca. 60 mg/ℓ) in medium and to the high nutrient treatments (Fig. 10).

Plant Biomass

Submerged Vascular Plants

Mean values for plant biomass prior to treatment were slightly higher in control ponds (ca. 120 gdw/m^2) compared to 80-87 gdw/m^2 among the other treatments (Fig. 11). Biomass increased in all treatments during June and July to levels ranging from a low of 157 gdw/m^2 in medium treatment ponds to a high of 237 gdw/m^2 in controls. Mid summer results also showed that *Ruppia* dominated the biomass of the control and medium treatments (71 and 63%, respectively) while *Potamogeton* dominated the low and high nutrient treatments (68 and 98%, respectively).

By August the mean total biomass had decreased in the medium and high nutrient ponds to about 80 gdw/m^2, similar to early summer values. Biomass in the low (196 gdw/m^2) and control (201 gdw/m^2) nutrient ponds were significantly higher ($p<0.05$) than values for the medium and high nutrient treatment.

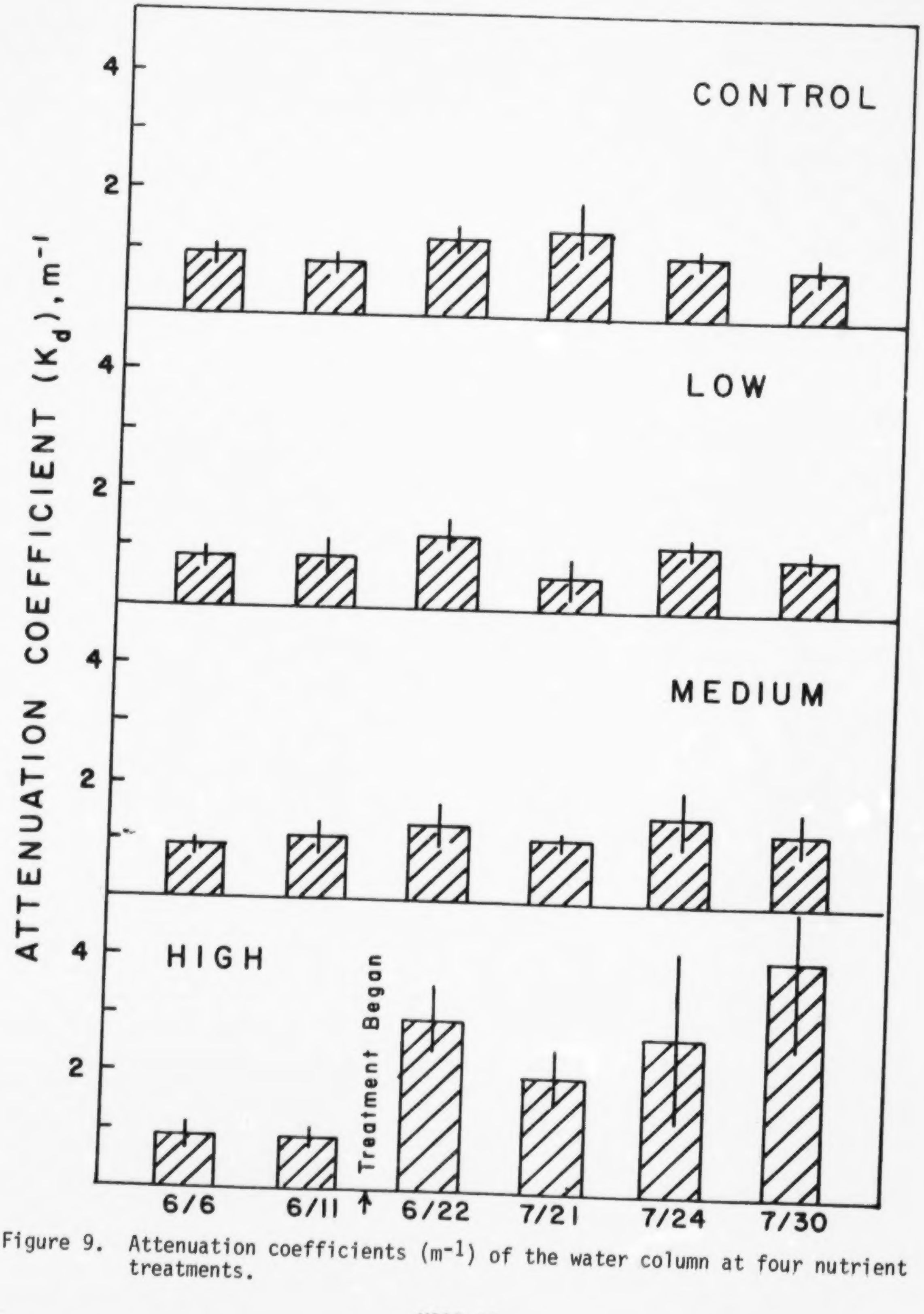

Figure 9. Attenuation coefficients (m^{-1}) of the water column at four nutrient treatments.

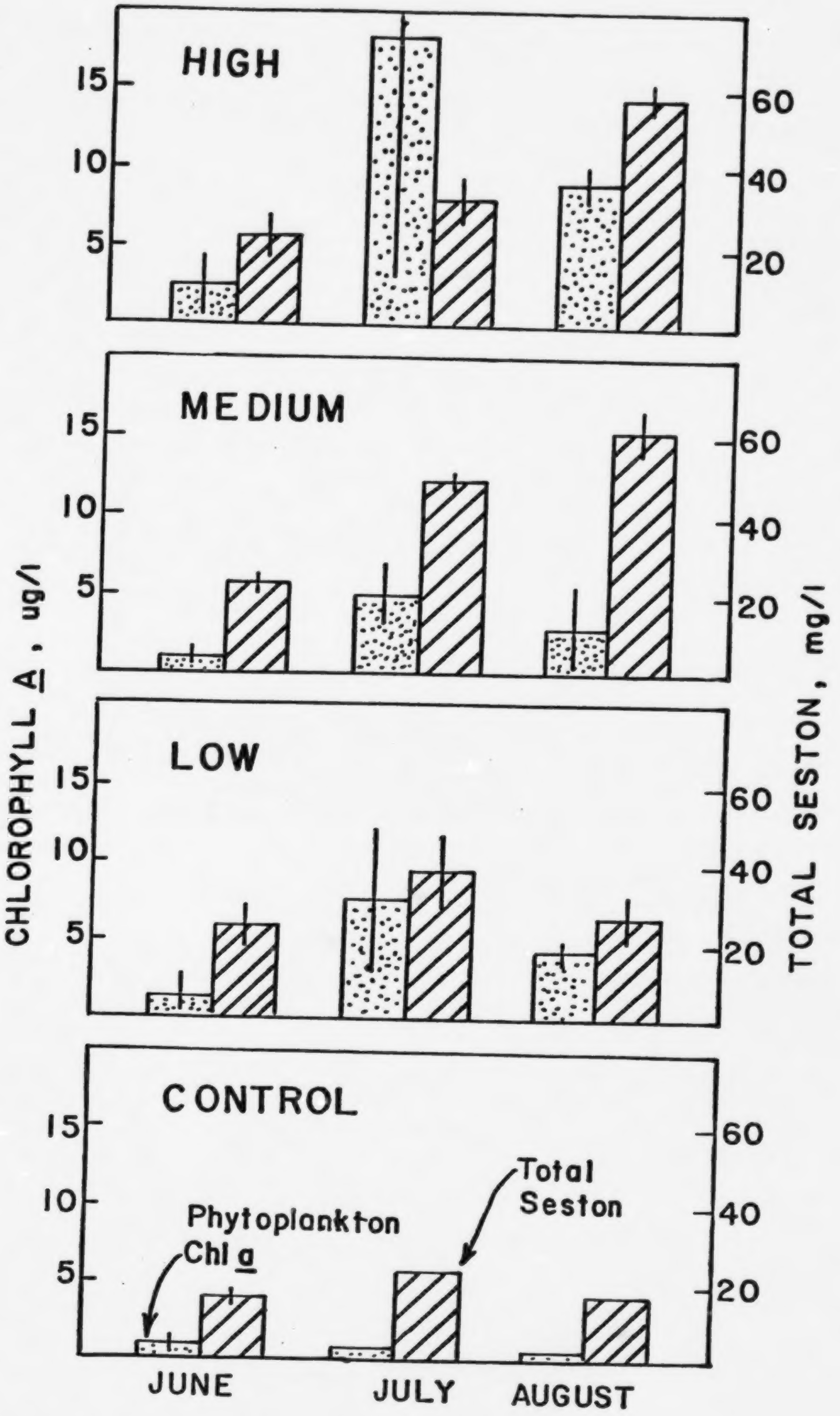

Figure 10. Concentrations of chlorophyll a (μg/ℓ) and total seston (mg/ℓ) in the pond waters at four nutrient treatments.

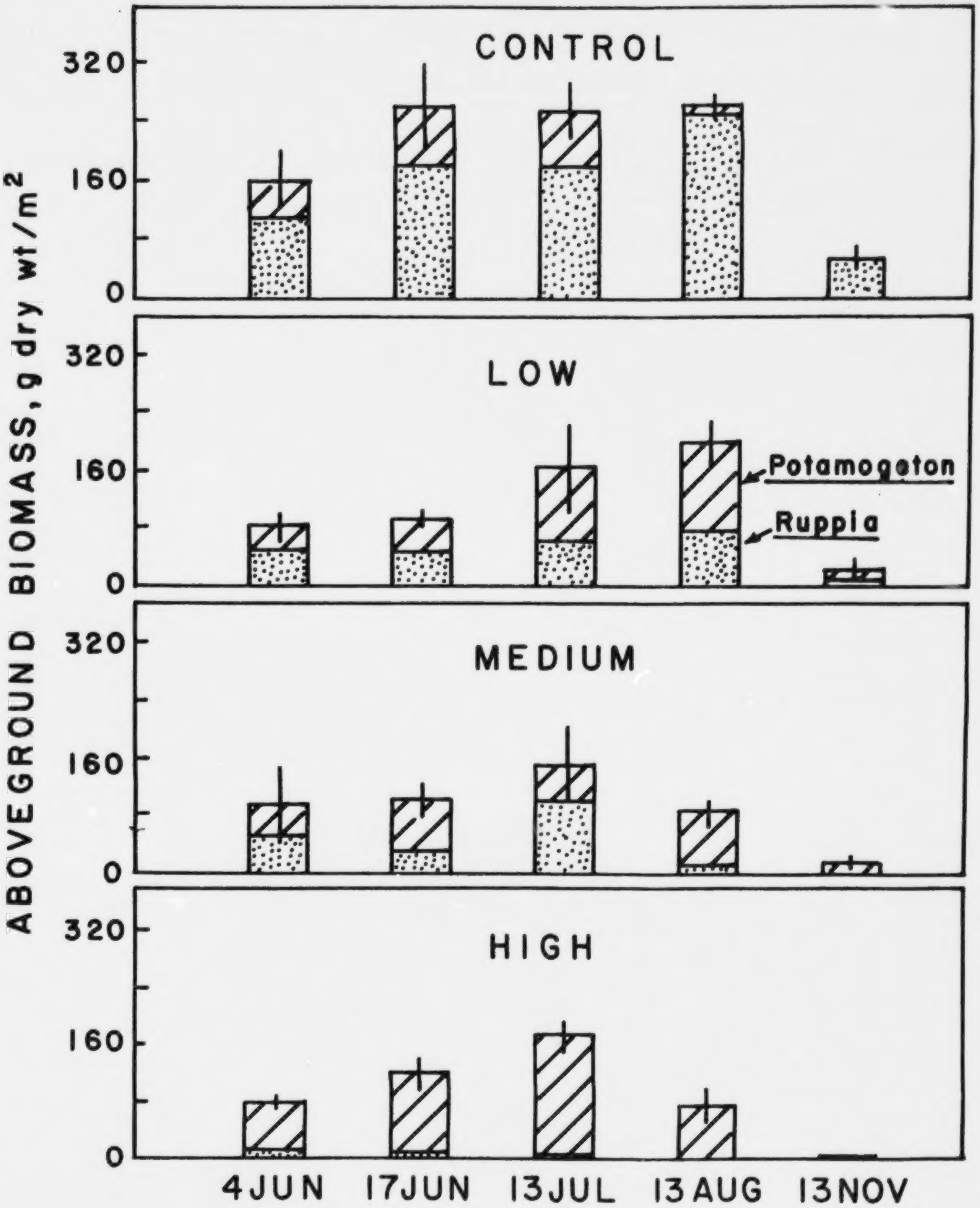

Figure 11. Aboveground biomass (gdw/m^2) of submersed macrophytes in ponds subjected to four nutrient treatments. Fertilization began on 18 June, 1981.

The biomass loss in medium ponds was marked by a decline in the presence of Ruppia from 63% of the total biomass in July (99.2 gdw/m^2) to only 7% of the total biomass in August (6 gdw/m^2). Ruppia was sparse in the high nutrient ponds in July (2% of the total) and absent in the high nutrient ponds in August. By November the effects of the fall senescent period on SAV biomass were evident in these systems, although all of the ponds except for those receiving high nutrient dose had some biomass remaining.

Changes in SAV standing crop in the ponds between sampling dates provide a measure of net productivity for these submersed grasses (Fig. 12b). There was an initial pulse of SAV production in the control pond followed by little growth during the remainder of the summer. In mid-June SAV net production ranged from 0.64 gdw $m^{-2} \cdot d^{-1}$ at the low nutrient treatment to 2.77 gdw$\cdot m^{-2} \cdot d^{-1}$ at the high nutrient treatment which follows the rank of nutrient levels applied. By mid-July net SAV production continued to increase in the low nutrient treatment, but declined slightly in the medium and high nutrient treatments to about 2.0 gdw$\cdot m^{-2} \cdot d^{-1}$. Net production of SAV in medium and high nutrient dosed ponds continued to decrease in August to values less the -2 gdw$\cdot m^{-2} \cdot d^{-1}$, while SAV net production in the low treatment was still positive at 1.2 gdw$\cdot m^{-2} \cdot d^{-1}$.

Since the stratified sampling strategy for SAV biomass did not monitor unvegetated areas of each pond, total biomass per pond using areal coverage of vegetation more clearly defines changes in SAV biomass in response to nutrient enrichment. Incremental changes in this total biomass in relation to initial pre-treatment measurements are given in Fig. 12a. Both control and low nutrient treatments exhibited nearly constant increases in SAV biomass during the summer, and increases in SAV biomass were also observed for medium and high nutrient treatments from early June to mid-July. However, from mid-July to mid-August the absolute biomass of SAV in both medium and high nutrient treatments declined. August biomass levels in medium nutrient ponds remained at 4 kg dry wt above early June levels, but SAV biomass was nearly 4 kg dry wt below the initial levels in high nutrient ponds. Therefore the biomass of SAV in the high nutrient pond showed a significantly greater negative response to nutrient enrichment.

Epiphytic Biomass

Dry weight and chl-a of total epiphytic material was sampled on three occasions during this experiment, once prior to treatment and twice during treatment. Epiphytic biomass as dry weight, was always highest for high and medium treated systems (Fig. 13), with mean values exceeding 3.7 gdw/gdw(plants). Low dose ponds exhibited a steadily increasing total epiphytic material, where mean values grew from about 1.2 in June to 2.4 gdw/gdw(plants) in August. Unfortunately, only one pond could serve reliably as control for this experiment. However, epiphytic biomass in this control pond remained at less than 5% of levels in all other ponds. This control system contained Ruppia maritima as the near exclusive SAV species, while the two high dose systems were virtually devoid of this species. However, there was no distinct pattern in the accumulation of epiphytes of R. maritima versus Potamogeton perfoliatus in the low and medium dosed ponds where both plants were important to total biomass (Figs. 11 and 13). Epiphyte levels were significantly greater for high nutrient

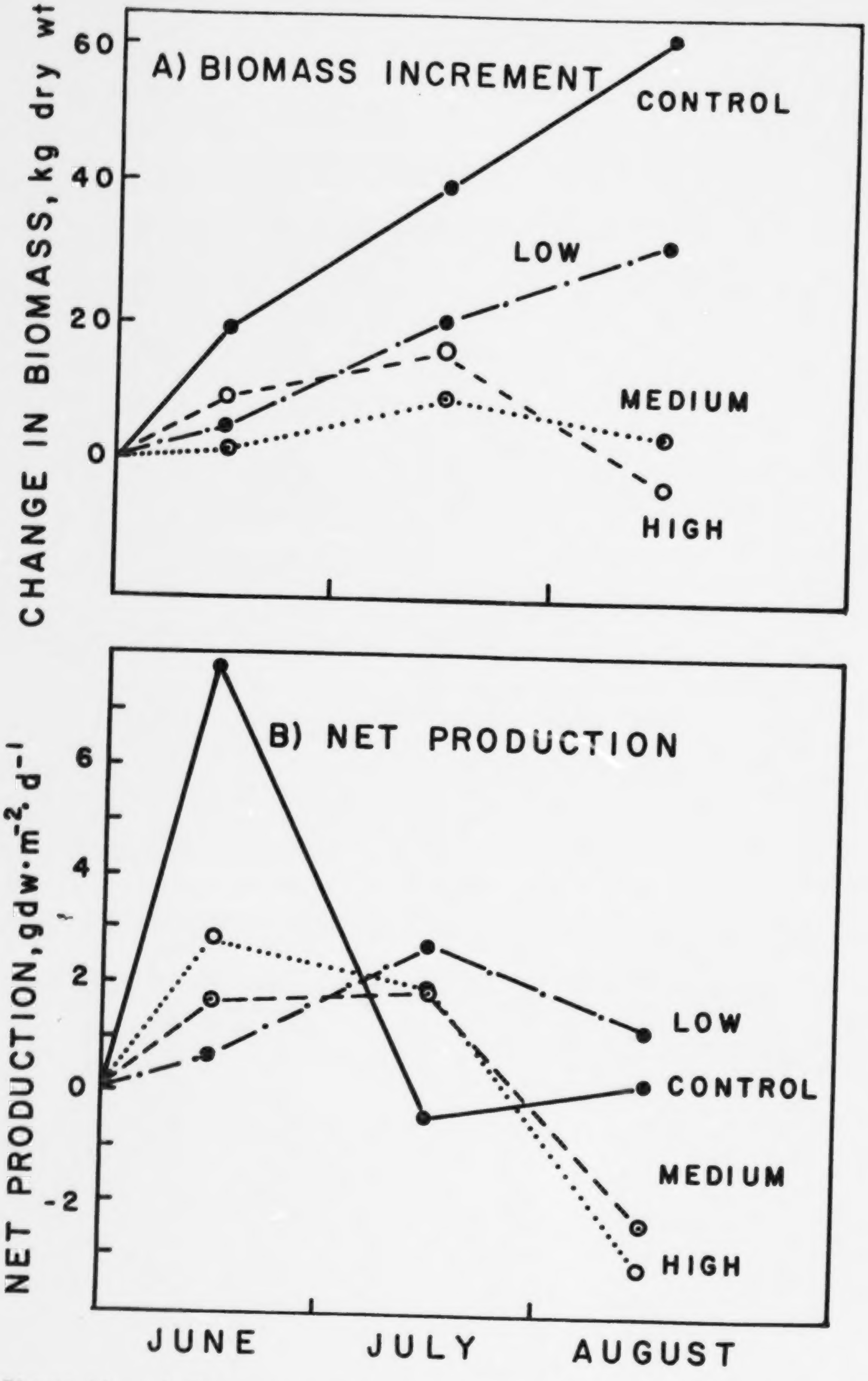

Figure 12. a) Changes in the total biomass of submersed macrophytes (kg dry wt.) in the ponds subjected to four nutrient treatments. b) Average daily changes in the biomass of submersed macrophytes gdw•m^{-2}•d^{-1}) in ponds at four nutrient treatments.

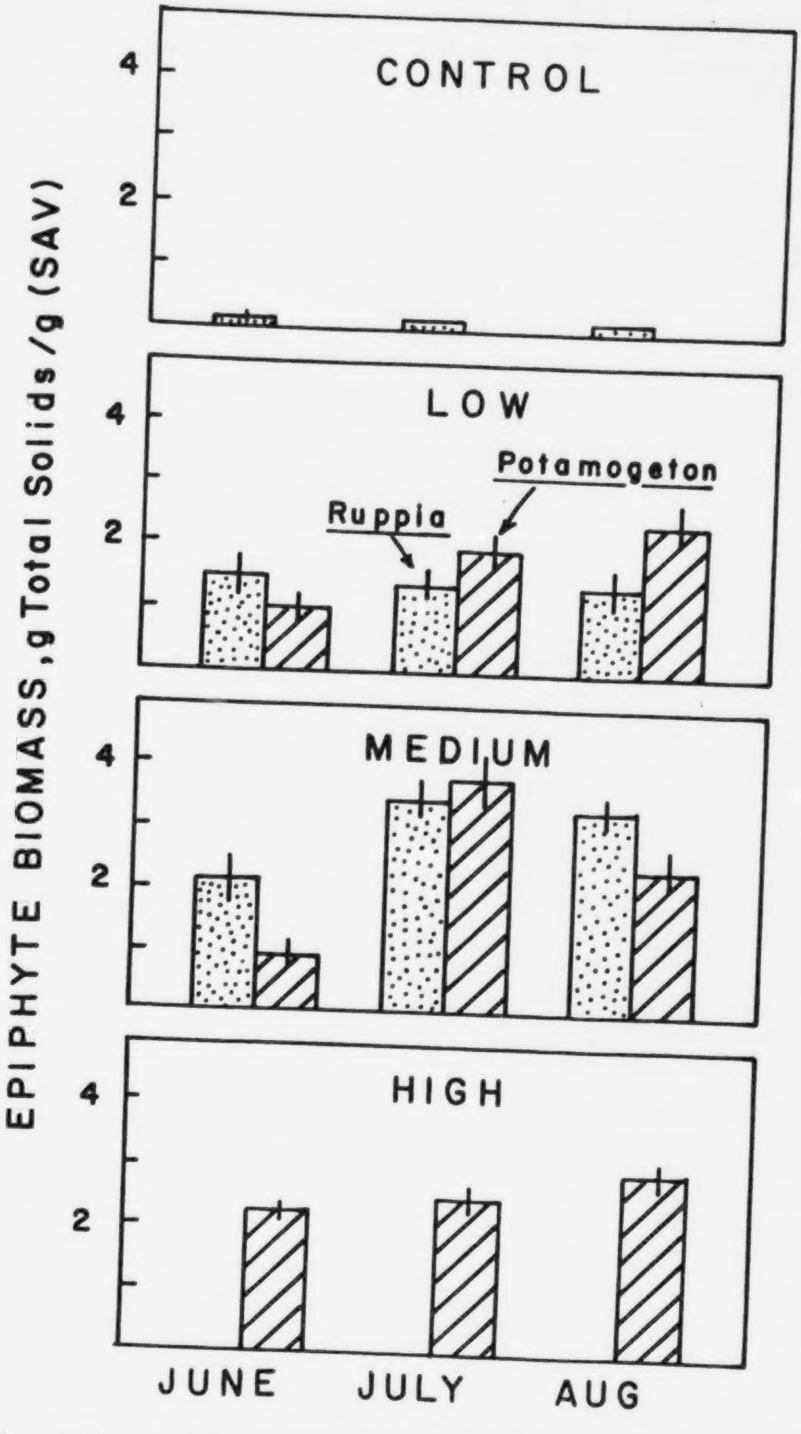

Figure 13. Epiphyte biomass (gdw/gdw(SAV)) on Ruppia maritima and Potamogeton perfoliatus in ponds subjected to four nutrient treatments. Fertilization began after the June measurements.

treatment than for low or control, whereas medium dosed epiphytes were significantly different only compared to controls.

Epiphytic chlorophyll-*a* biomass shows a much clearer response to nutrient treatment, although data from a control pond (#1) was not considered (Fig. 14). Chl-*a* measurements were less than 0.3 mg/gdw(plants) at pretreatment for both species. By August, chlorophyll-*a* remained at this pretreatment level on *Ruppia* in the control pond, while concentrations on *Ruppia* and *Potamogeton* increased in relation to nutrient treatment up to 1.6 mg/gdw(plant) in the high nutrient pond (*Potamogeton*) (Fig. 14). The most dramatic increase in chlorophyll-a occurred on *Potamogeton* in the high nutrient applications of nutreints. The chlorophyll-*a* biomass increased from 0.07 to 1.58 mg/gdw in one month. This increase represented a shift in the chlorophyll-a: total solids ratio from 0.003 to 0.054, compared to a change of about 0.012 to 0.018 in the other two treatments for *Potamogeton*.

Plant and Epiphyte Productivity

Plant Production and Respiration

Diurnal patterns of apparent production of *P. perfoliatus* (Fig. 14) show unvarying rates from 1100 - 1600 h on a cloudless summer day (15 June 1981). Production:respiration ratios (P/R) were also remarkably constant during this period at about 1.8. In contrast, apparent production rates of *R. maritima* exhibited a distinct temporal sequence, with rates at midday nearly 4 times greater than morning and afternoon rates, which were similar. Respiration, however, was constant during the day for *R. maritima*, resulting in a range of P/R ratios from 1.8 in the morning to 5.7 at midday. The peak apparent production rate for *Ruppia* was 10.2 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ compared to 8.1 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ for *Potamogeton*.

Vertical distribution of production along plant stems was also different between these two species (Fig. 16). Net production varied linearly along the length of *Potamogeton* ranging from 5 mg $C \cdot gdw^{-1} \cdot h^{-1}$ for the top 5 cm section to 1.2 mg $C \cdot gdw^{-1} \cdot h^{-1}$ for the bottom section, more than 45 cm from the tip (Fig. 16). However, net production was nearly constant along the length of *Ruppia* at about 3.5 mg $C \cdot gdw^{-1} \cdot h^{-1}$. The quantity of PAR available from the water surface to 0.5 m is also shown in Fig. 16, and indicates that it was in excess of saturation values for photosynthesis (300-600 μ Ein $m^{-2}s^{-1}$) for both *Potamogeton* and *Ruppia*.

The internodal length (i.e., distance between leaf rodes on the stem) decreased along *Potamogeton* from less than 1.0 cm in the terminal 10 cm of the plant to greater than 2 cm at the base. Since the meristem of *Potamogeton* is terminal, the leaves at the tip are usually younger than leaves in the basal section. The branching of *Ruppia* is more consistent along the length of the plant and these branches, even those near the base of the plant, are long enough to reach the surface of the water. Therefore the net productivity dissimilarities of these two submerged species seems to be a result of the morphological arrangement of the photosynthetic structures along the stem of the plants.

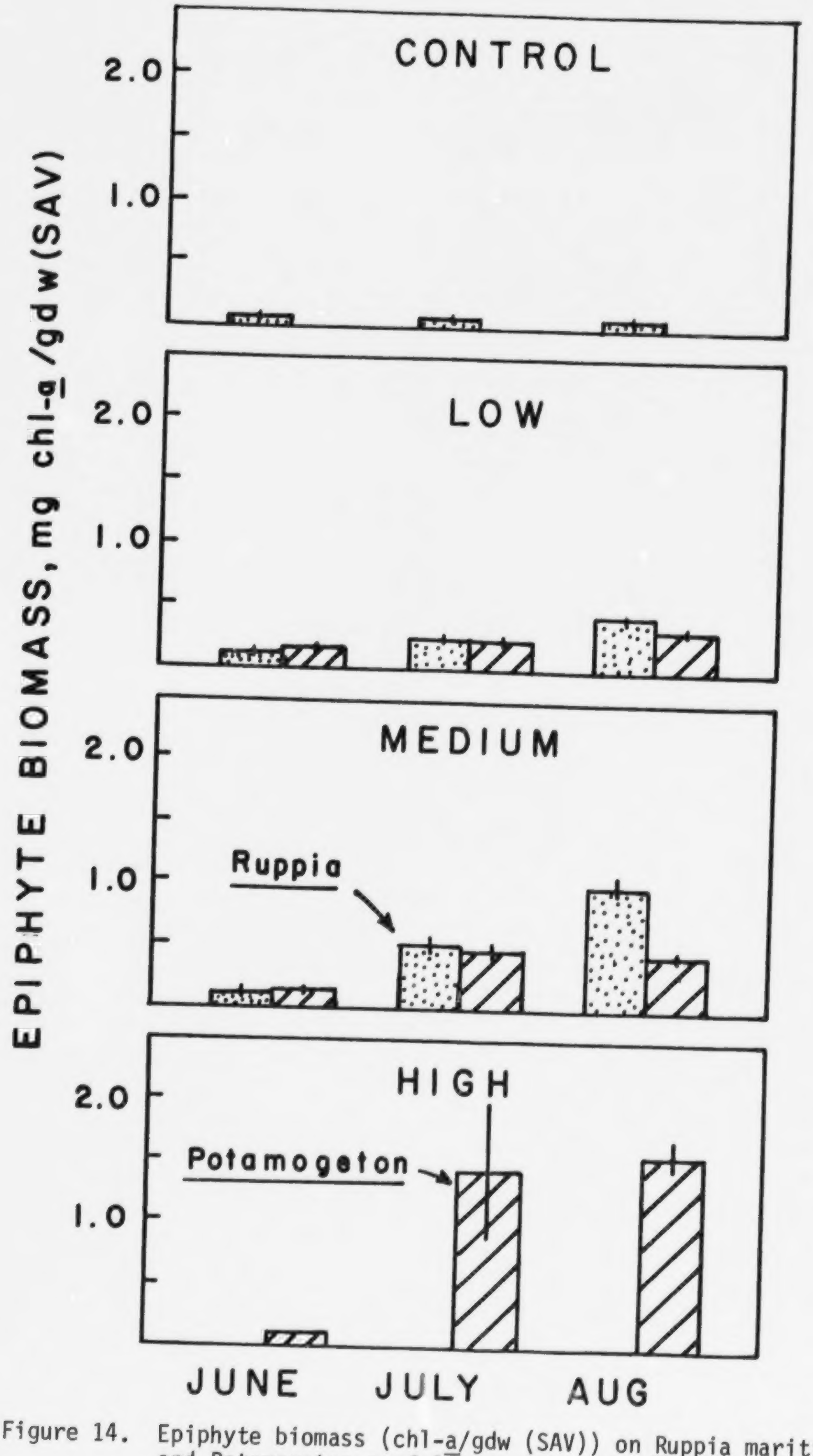

Figure 14. Epiphyte biomass (chl-a/gdw (SAV)) on Ruppia maritima and Potamogeton perfoliatus in ponds subjected to four nutrient treatments. Fertilization began after the June measurements.

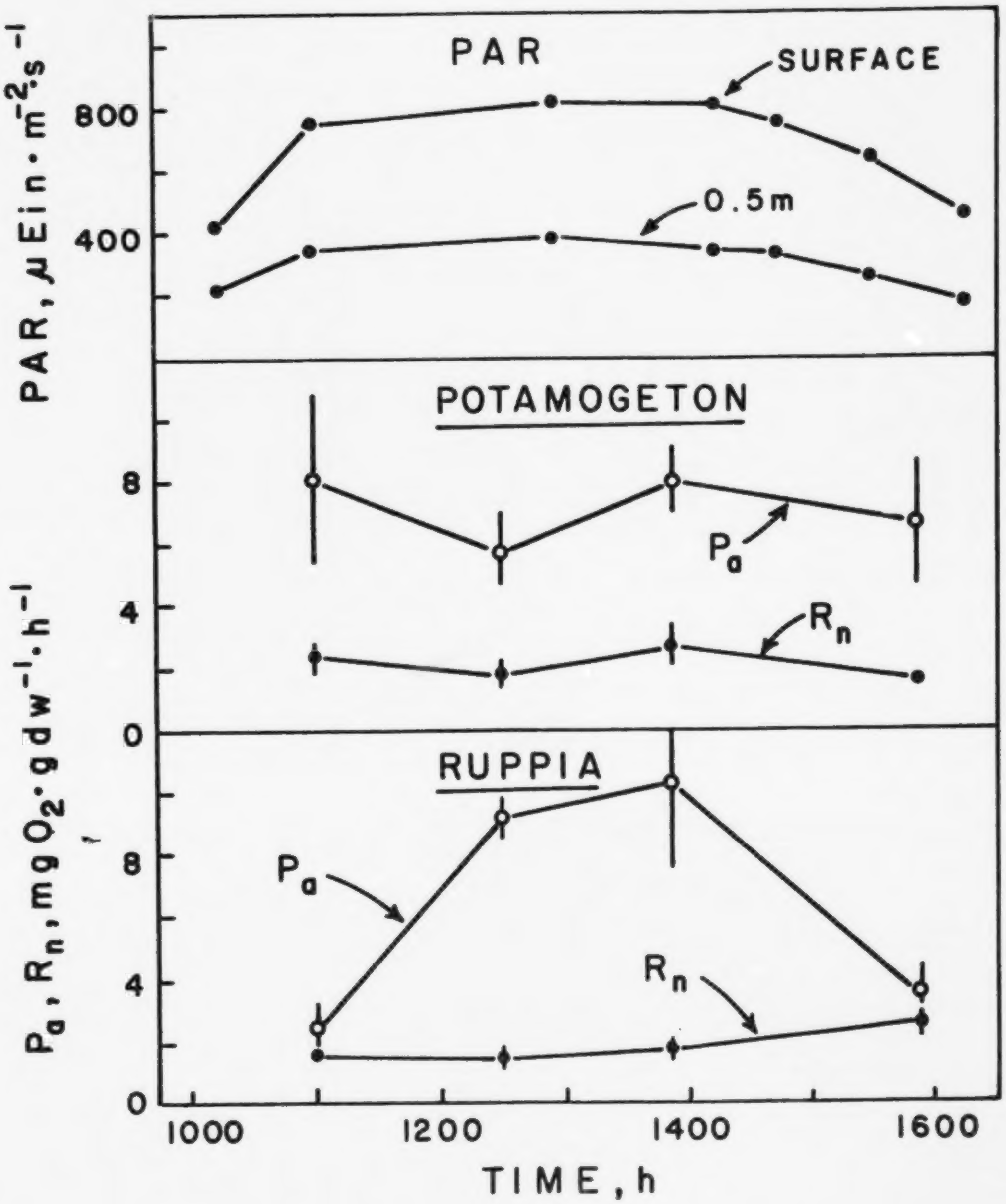

Figure 15. Photosynthetic active radiation (µEin•m^{-2}•s^{-1}) at the water's surface and at 0.5 m depth from midmorning to midafternoon along with measurements of apparent production (P_a) and net respiration (R_n) for Ruppia maritima and Potamogeton perfoliatus.

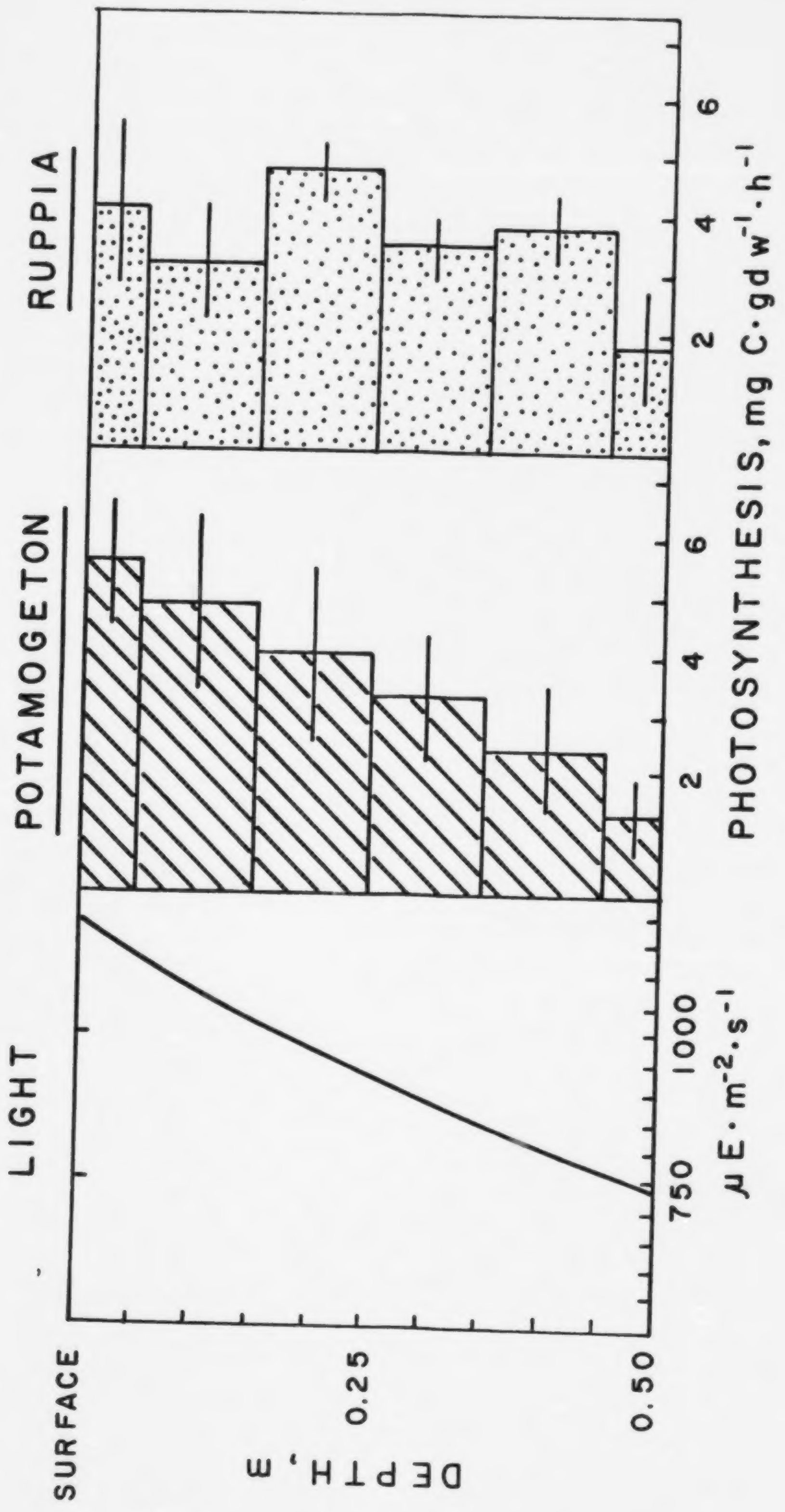

Figure 16. Net photosynthesis (mg C•gdw^{-1}•h^{-1}) along the length of Potamogeton perfoliatus and Ruppia maritima cleared of epiphytic material. Included is the amount of photosynhtetic active radiation (μEin•m^{-2}•s^{-1}) from water's surface to 0.5 m depth.

Plant/Epiphyte Relations

Apparent production of *Potamogeton* taken from a low nutrient pond, was greater than 5 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ for the tip sections, with rates above 8.1 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ on two dates for plant tips cleaned of epiphytic mater (Fig. 17). P_a of the basal sections of *Potamogeton* was always lower than rates for the tip ranging from 4.8 to 6.0 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ (55% of P_a for the 0-10 cm sections). The combined P_a of plant and epiphytes ranged from 11.2 to 12.4 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ for apical portions, and 5.8 to 7.2 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ for lower stem. In all cases, plants dominated total apparent production in the low nutrient pond. Epiphyte levels on *Potamogeton* in this pond were less than 2.0 gdw/gdw(SAV).

Peak P_a for *Potamogeton* from high nutrient ponds was only 4.0 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ (Fig. 17). This rate was for a tip section of the plant which had 1.40 gdw/gdw(SAV) of epiphytes, which was the lowest epiphyte biomass value observed on the plants tested in this pond. At epiphyte biomass levels above 3.0 gdw/gdw(SAV), SAV production was less than 1.0 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$, which occurred for both the tip and basal sections of *Potamogeton* in August. Although the contribution of SAV to total production was minor, the elevated epiphyte biomass resulted in total P_a (epiphyte plus SAV) $>$ 5 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ for July and August with a peak value of 8.0 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$. The contribution of epiphytes to this production ranged from 58 - 100%.

SAV respiration ranged from 0.30 to 2.45 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ with an average of 1.4 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ for *Potamogeton* from the low nutrient treated pond resulting in P:R ratios from 4.0 to 30. P:R ratios for epiphytes from this pond ranged from 0 to 3.6; therefore the total production of the SAV/epiphyte community was always greater than total respiration at an average ratio of 4.4 (Table E-1). Respiration of *Potamogeton* from the high nutrient treatment ranged from 0.40 to 6.03 mg $O_2 \cdot gdw^{-1} \cdot h^{-1}$ with an average of 1.9 g $O_2 \cdot gdw^{-1} \cdot h^{-1}$. These higher respiration rates together with reduced net production values resulted in much lower P:R ratios ranging from 0 to 9.8. Only 2 of the 5 measurements of SAV metabolism from the high nutrient treatment had P:R ratios greater than one. Although the P:R ratios of epiphytes from this treatment were similar to those ratios for epiphytes from the low nutrient pond; the total P:R ratio of the SAV/epiphyte community under high nutrient application was only 1.8 compared to 4.4 for the low nutrient treatment.

These data are based on DO changes of epiphytes in one chamber and epiphytes plus SAV in the other. By subtracting these results, the partitioning of production and respiration of just SAV and epiphytes were calculated for each experiment. The uptake of $^{14}C\text{-}CO_2$ by epiphytes and SAV gives a more direct measurement of the relative net productivities of these plants under varying levels of epiphyte biomass.

Net production (as $^{14}C\text{-}CO_2$ incorporation) for the 0-15 cm section of *Potamogeton* decreased to 73% of control values at an epiphyte density of 5.04 gdw/gdw(SAV). However, the total net production of the epiphyte and SAV

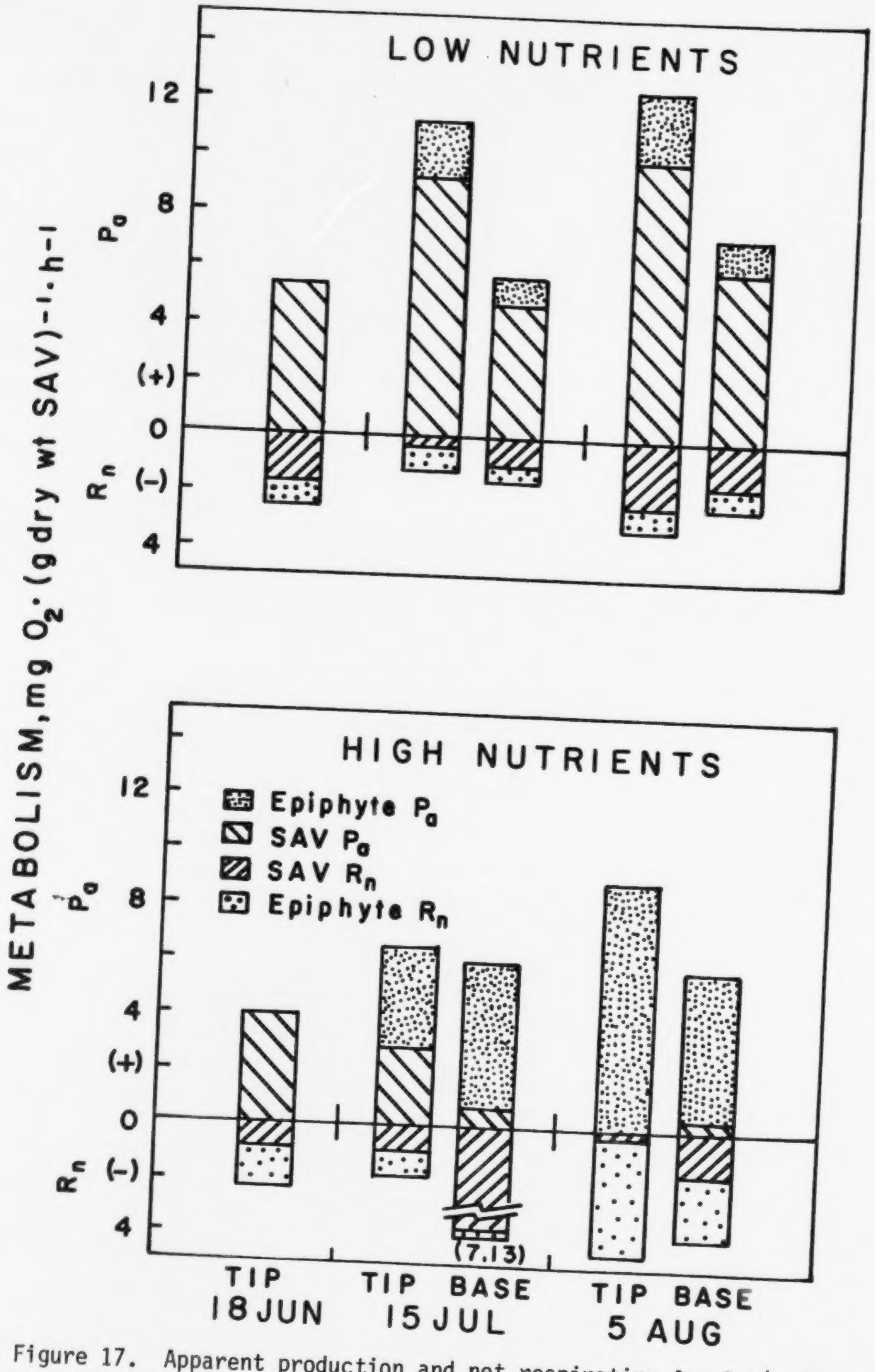

Figure 17. Apparent production and net respiration [mg O_2 (g dry wt. SAV)-1·h-1] of Potamogeton perfoliatus and associated epiphytes from a low nutrient and a high nutrient treated pond.

community increased from 5.09 mg $C\cdot gdw^{-1}\cdot h^{-1}$ (control) to 7.79 mg $C\cdot gdw^{-1}\cdot h^{-1}$ at this high epiphyte biomass level (Fig. 18). This was an increase of 153% of the net production of nonepiphytized plants. At the same epiphyte biomass level, net production of the 15-55 cm section of *Potamogeton* decreased to only 56% of the control. As observed for the tip of *Potamogeton*, the basal section of the SAV/epiphyte community increased in total net production from 2.32 mg $C\cdot gdw^{-1}\cdot h^{-1}$ (control) to 3.78 mg $C\cdot gdw^{-1}\cdot h^{-1}$ (163%). At medium levels of epiphyte biomass, which ranged from 1-2 gdw/gdw(SAV), the net production of neither the tip nor basal sections of the plant were affected by the fouling.

Epiphyte biomass was much lower on *Ruppia* compared to *Potamogeton* at 0.49 and 0.39 gdw/gdw(SAV) for tip and base, respectively. However, the reduction in photosynthesis on these plants was much higher at 67% of the control for the tip and only 23% of the control for the basal sections. Also in contrast to *Potamogeton*, the total community net production of the lightly fouled *Ruppia* was lower than the controls. Therefore epiphyte production was not enough to compensate for the reduced net production of *Ruppia* due to epiphytic fouling.

Community Photosynthesis and Respiration

Apparent production in control and low nutrient pond communities exhibited similar patterns with an increase in net production during the latter week of July followed by a slight decrease in mid-August (Fig. 19). The peak production rates in these two treatments were 8.71 and 8.23 g $O_2\cdot m^{-3}\cdot d^{-1}$, respectively. Through June and mid-July the medium and high nutrient dosed ponds also increased in net production with peak values of 11.05 and 12.93 g $O_2\cdot m^{-3}\cdot d^{-1}$, considerably higher than for control and low treatments. The peak increase in net production rates in these two treatments was about 4.5 g $O_2\cdot m^{-3}\cdot d^{-1}$ above pretreatment values, and the increase occurred sooner in the high dose pond compared to medium nutrient treatment. This rate of change in net production during the summer was more than double the increases observed in both the control and low nturient treatment (Fig. 20).

Following these late July peaks, community P_a decreased in the control, low, and medium nutrient treatments by similar magnitude during August. However, only the medium nutrient pond had net production values greater than pretreatment values. Net production in the high nutrient ponds declined drastically during this period to levels more the 3.0 g O_2 $m^{-3}\cdot d^{-1}$ less than pretreatment values. This decline in net production represented a total decrease of more than 7 g$O_2\cdot m^{-3}\cdot d^{-1}$ during a 10-day period, and it is coincident with the observed loss of SAV. Only under high nutrient dosaged did P:R become less than 1.0. From the end of July to mid-August the P:R ratio of these ponds dropped from 1.5 to 0.6.

DISCUSSION

Nutrient Uptake from the Water Column

Uptake rates of dissolved inorganic nitrogen and phosphorus from the

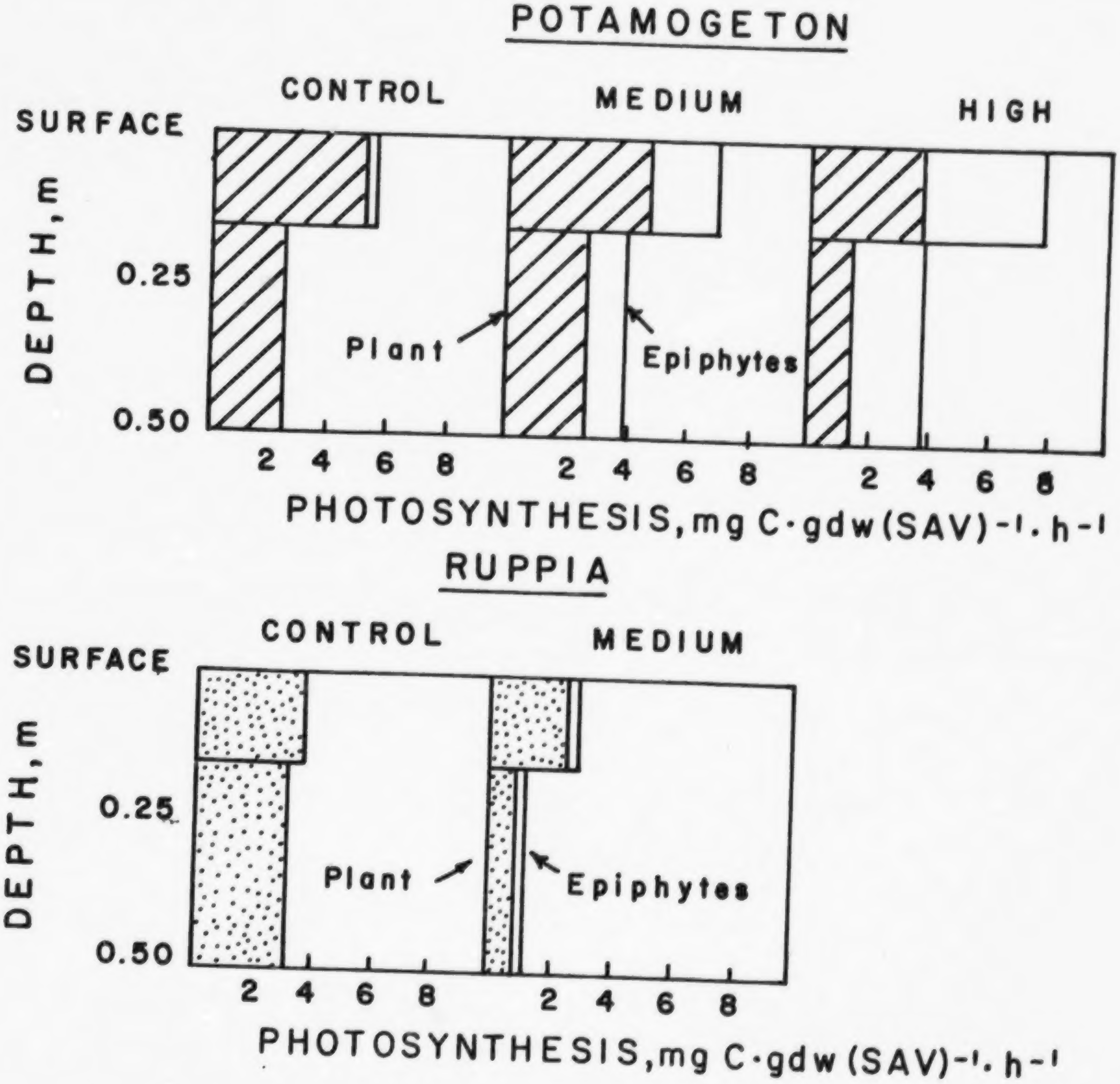

Figure 18. Net photosynthesis [mg C (g dry wt. SAV)$^{-1}$·h^{-1}] of the tip (0-15 cm) and basal (15-50 cm) sections of Potamogeton perfoliatus and Ruppia maritima and associated epiphytes.

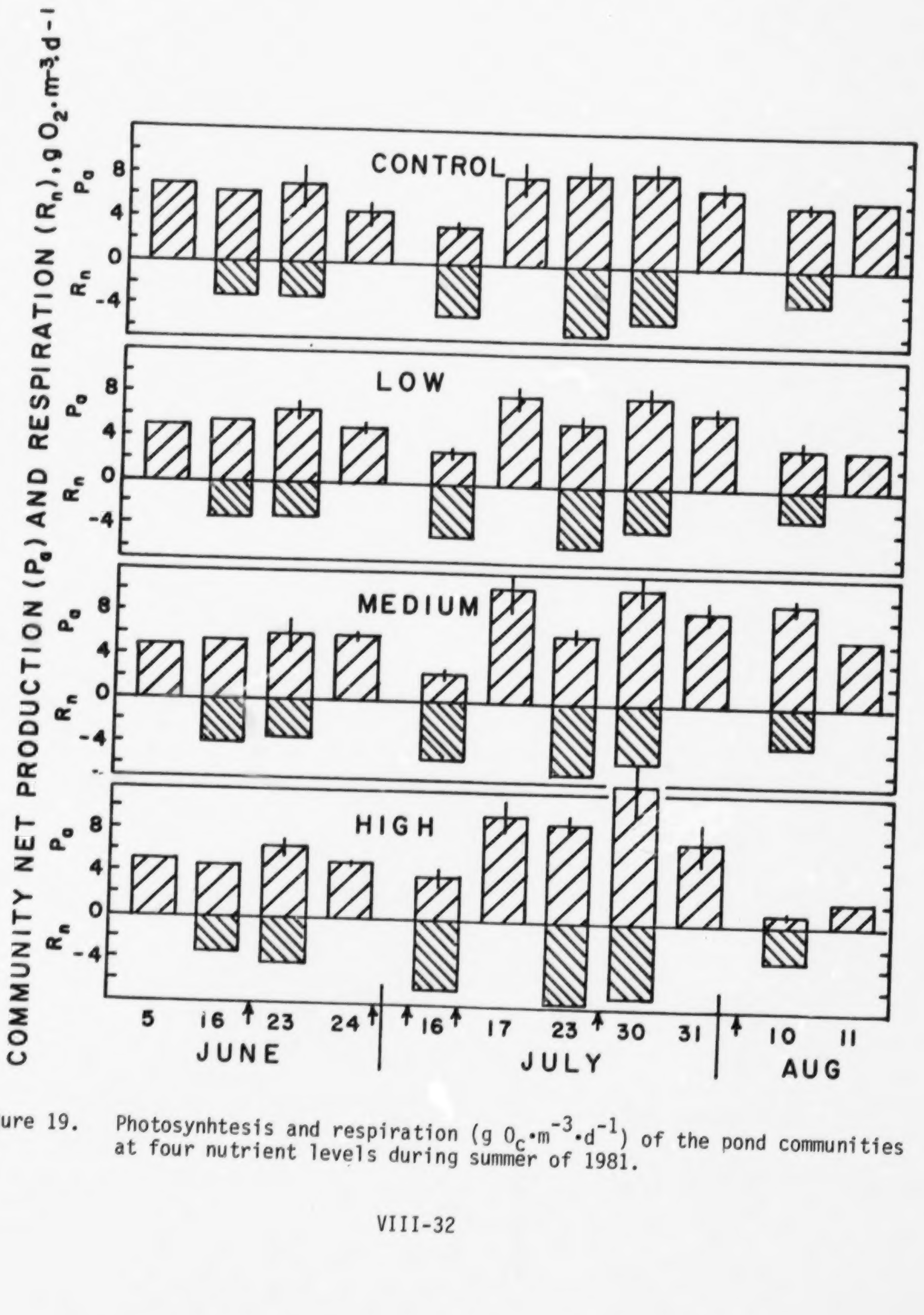

Figure 19. Photosynhtesis and respiration (g $0_c \cdot m^{-3} \cdot d^{-1}$) of the pond communities at four nutrient levels during summer of 1981.

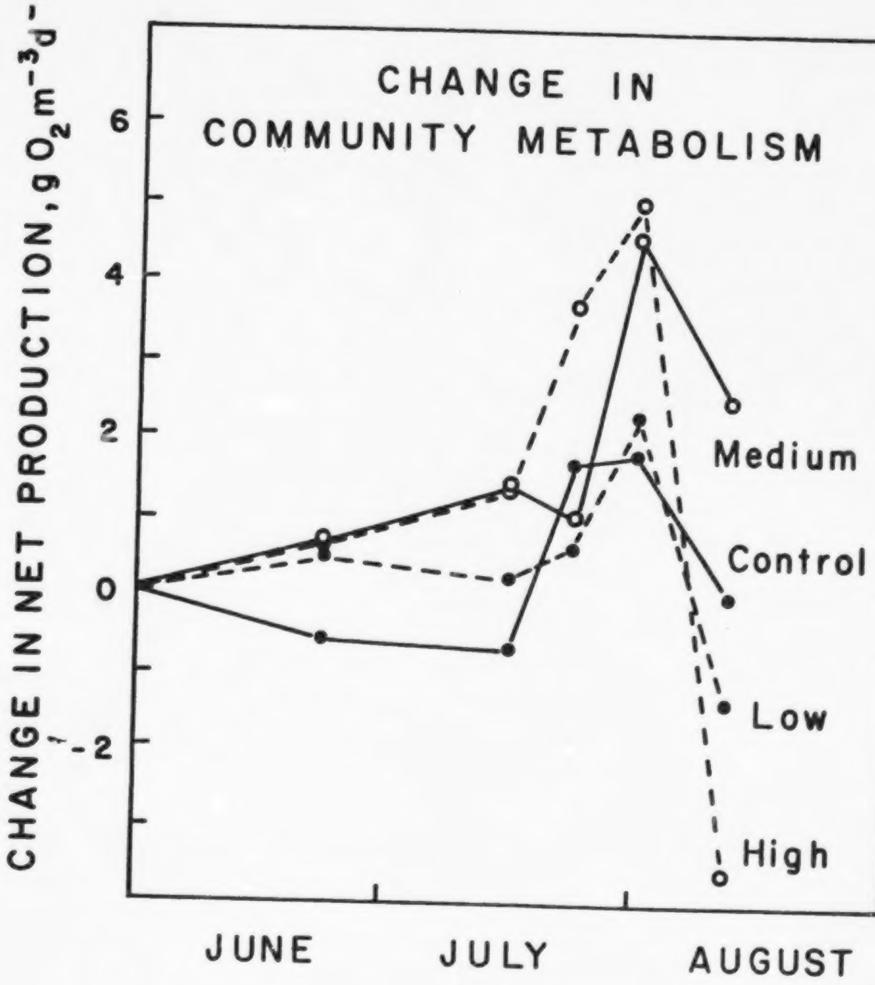

Figure 20. Changes in net production (g $O \cdot m^{-3} \cdot d^{-1}$) of the pond communities at four nutrient treatments during the summer of 1981. Fertilization began after the June measurements.

water column appear to have been dependent on concentration (Fig. 21). Uptake of NO_3^- and NH_4^+ were similar between 0 and 10 μM N; above this concentration NH_4^+ uptake rates were much higher, indicating a preference for NH_3^+. At about 60 μM N the uptake was only 18 μM/d for NO_3^- compared to 30 μM/d for NH_4^+. The curves in Fig. 21 suggest that NO_3^- and NH_4^+ uptake by these SAV communities was diffusion-limited without clear indication of saturation up to 90 μM. Other investigators have, also, reported a preference of NO_3^- over NH_4^+ for various SAV species (Nichols and Keeney 1976; Best 1980; Iizumi and Hattori 1982) as well as phytoplankton (McCarthy et al. 1976).

These NO_3^- and NH_4^+ uptake curves are similar to patterns of nutrient uptake observed elsewhere for P. perfoliatus. Laboratory uptake studies have shown that NH_4^+ uptake by shoots of P. perfoliatus is 10 times greater than NO_3^- uptake (Stevenson et al. in prep). Field studies in P. perfoliatus beds along the Choptank River estuary using 0.51 m deep chambers revealed that NH_4^+ uptake rates were similar to those for NO_3^- at N concentrations up to about 30 μM, but at concentrations > 75 μM, NH_4^+ uptake rates were generally twice those of NO_3^- (Kaumeyer et al. 1981). Thus, it appears that SAV communities have a higher absorption capacity for NO_3^- and NH_4^+ compared to plankton communities, and that the absorption rates for NH_4^+ are much higher compared to NO_3^- uptake.

Nitrogen Uptake and Storage by SAV

The relationship between tissue nutrient content of aquatic plants and nutrient enrichment observed in this study has two important ecological implications. Tissue nutrients in aquatic plants have been proposed as an indicator of the nutrient status of their ecosystem (Gerloff and Krombholz 1966; Fitzgerald 1969; Gossett and Norris 1971; Adams et al. 1971, 1973; Seddon 1972). Also, aquatic plant communities may be managed in a variety of schemes for the treatment of nutrient wastes with subsequent economic benefits from the increased nutritional quality of the plants (Boyd 1970, 1971; Siedel 1971; McNabb 1976; Wolverton et al. 1975).

In this study, N content of Potamogeton leaves and stems increased markedly in proportion to nutrient enrichment. The maximum tissue N concentration (3%) was observed in the high nutrient treatment (120 μM N/wk). In contrast, tissue N concentration of Potamogeton growing in ambient estuarine waters was only 1.2% N. Nitrogen content of plants at low and medium treatments were intermediate, both being about 2% N.

Boyd and Scarsbrook (1975) also observed significant increases in tissue N of Eichhornia crassipes for fertilized ponds; however, there was little distinction in tissue N (1.8%) among various nutrient enrichments ranging from 7 to 35 μM N per week. Under laboratory conditions, media concentrations of 3000 μM N were required for the blades and floats of E. crassipes to reach N concentrations of 2.0% (Gosset and Norris 1971). A change in N content for Vallisneria americana from 1-2% in the laboratory occurred in response to an increase from 500 to 2000 μM (as NO_3^-) in the growth medium (Gerloff and Krombholz 1966). Our study indicates that P. perfoliatus with tissue N > 1.5% may be an indication of a nutrient enriched environment, although precise

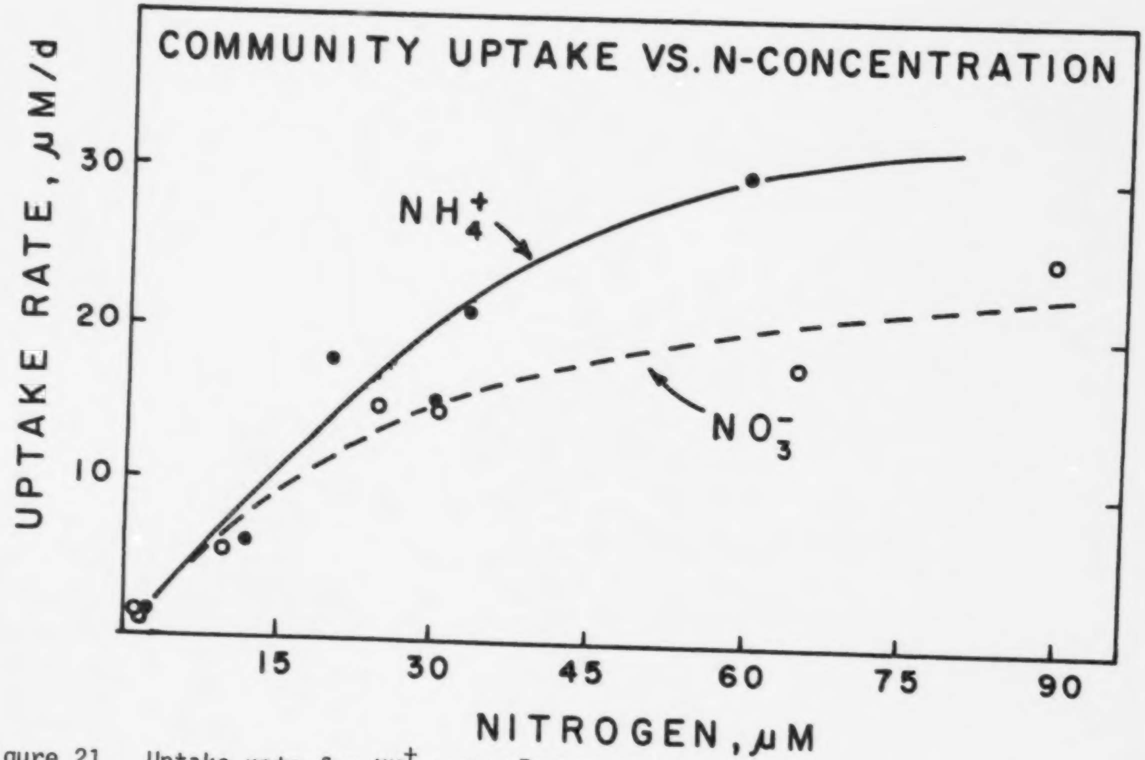

Figure 21. Uptake rate for NH_4^+ and NL_3^- from pond waters with submersed macrophyte communities at various concentrations of nitrogen

relationships to the "trophic" status of those waters await further study.

Luxurious accumulation of nutrients in submersed aquatic plant communities has been used successfully in ameliorating nutrient waste problems (Sinclair and Forbes 1980). Gerloft and Krombholz (1966) showed that *V. americana* accumulated nearly 75% of the N in growth media ranging from 150 to 3000 µM N, while at 6000 µM the percent recovery dropped to 56%. During their study, plant growth rates remained relatively constant while N content of plants increased. In our experiment, both SAV production and tissue N increased with fertilization (Fig. 22). It appears that growth was stimulated in associated with tissue N >1.5%, which is similar to the critical concentration of 1.3% N proposed by Gerloff and Krombholz (1966). Nutrient amended plants accumulated about 1.45 g N/m^2 more than plants in unfertilized ponds in July. This excess accumulation was due to increased tissue nitrogen and biomass production for all nutrient treated ponds (Table 1). The percentage of applied nitrogen that was recovered in SAV biomass ranged from 113% in the low nutrient ponds to 30% for the high nutrient treatment (Table 1). By 13 August following six applications of nutrients to the ponds, the percent recovery of the applied N by SAV tissue changed dramatically. Results for the low nutrient treatment were similar to that observed on 13 July (127% recovery, Table 1). However, N incorporation in the medium and high nutrient ponds dropped to 9 and 7%, respectively. Similar results were observed in pond fertilization studies with *E. crassipes*, where at 4 and 8 µM N treatments the plants recovered large amounts of N; but at the 34 µM N treatments recovery was minimal (Boyd & Scarsbrook 1975).

This change in the pattern of N accumulation by SAV can be explained by examining the relationship between tissue N concentrations and SAV biomass production on 13 August (Fig. 22). Low nutrient treatments resulted in an increase in biomass production and an increase in tissue N concentrations to 1.8% in July. However, even though tissue N concentrations were greater than 2.0% in the high nutrient ponds, SAV production was negative by as much as 3.0 $gdw \cdot m^{-2} \cdot d^{-1}$ (Fig. 22). In short-term laboratory studies where plankton populations are controlled, SAV growth is observed for all nutrient amendments. In these field experiments, SAV biomass decreased under highly eutrophic conditions. Therefore, eventhough the tissue N of SAV may increase in response to nutrient enrichment; the "nutrient buffering effect" of SAV is operative only under moderate eutrophic environments.

The luxuriant consumption of nutrients by aquatic plants may not only ameliorate moderate eutrophic conditions; but also increases the nutritional quality of the macrophytes. In this study, an increase in nutrient enrichment resulted in a proportional decrease in tissue C:N ratios of SAV. Presumably this inorganic nitrogen was converted to amino acids and proteins which would increase the value of SAV as food (direct or indirect) for fish and waterfowl. For macroalgae some of this excess tissue N may be stored as NO_3^- rather than protein (Wheeler and North 1981). Although it has been shown that seasonally elevated tissue N is usually proteinaceous (Best 1977), further studies are needed to determine the chemical nature of accumulated N in SAV in response to fertilization.

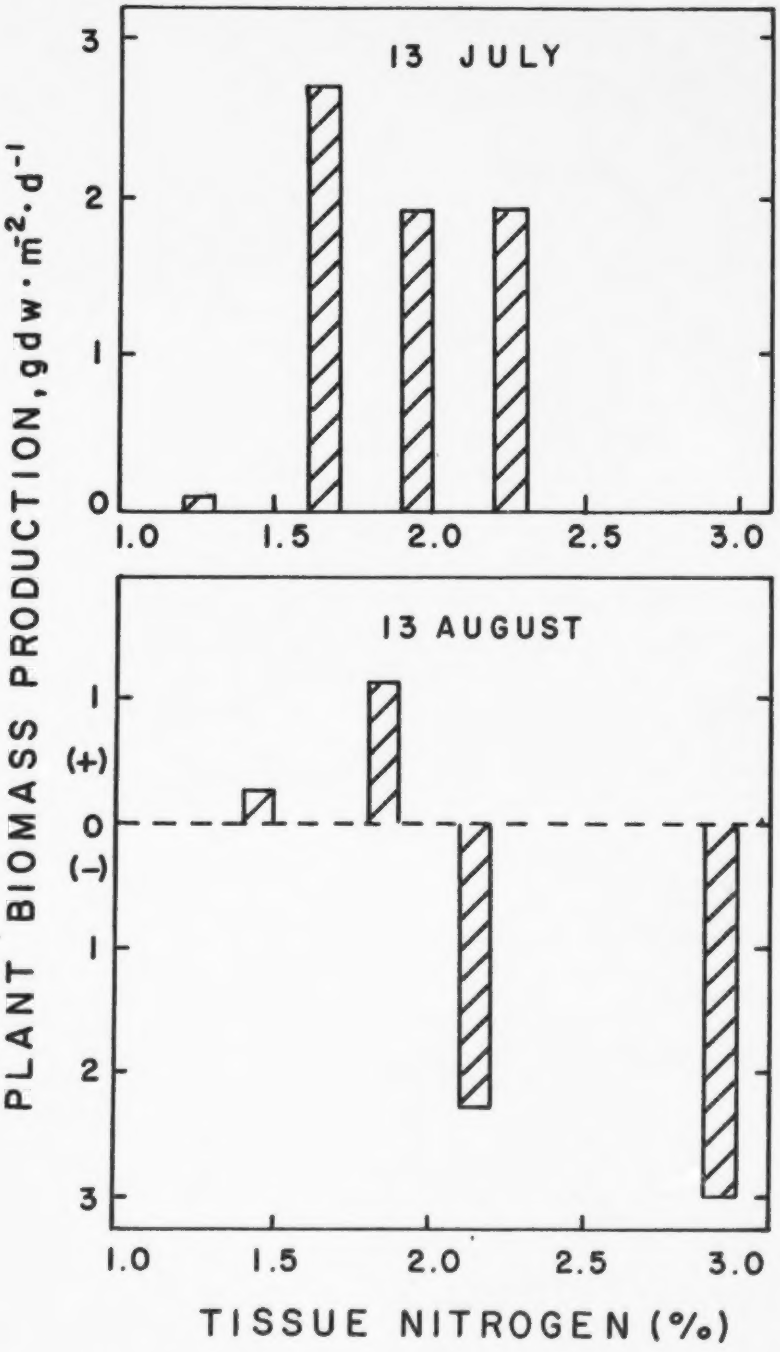

Figure 22. The relationship between tissue nitrogen content (%) and biomass production of submersed macrophytes (gdw·m^{-2}·d^{-1}).

Table 1. The amount of nitrogen applied (g/m^2) compared to the amount of nitrogen recovered in plant tissue (g/m^2) above controls for three nutrient enrichment treatments.

	NUTRIENT ENRICHMENT		
	LOW	MEDIUM	HIGH
13 July			
Nutrients Applied (g/m^2)	1.26	2.52	5.04
Nutrients Recovered in SAV (g/m^2)	1.42	1.40	1.49
% Recovery	113	56	30
13 August			
Nutrients Applied (g/m^2)	2.52	5.04	10.08
Nutrients Recovered in SAV (g/m^2)	3.19	0.47	0.72
% Recovery	127	9	7

Sediment Nutrient Dynamics

Denitrification rates measured in the low (~20 mol N m^{-2} h^{-1}) and high (~40 μmol N$\cdot$m$^{-2}\cdot$h^{-1}) nutrient treatments were within the typical range of 10-100 μmol N$\cdot$m$^{-2}\cdot$h^{-1} previously reported for denitrification in estuarine sediments (Sorsenson 1978; Koike and Hattori 1979; Oren and Blackburn 1979; Seitzinger et al. 1980; Henrikson et al. 1981; Nishio et al. 1982). Denitrification rates in our study responded to NO_3^- enrichment; however while NO_3^- levels were increased by a factor of 7 (10-70 μM), the denitrification rates only doubled. This is consistent with the kinetic relations reported by Billen (1978). Koike and Hattori (1979) and others who indicate that K_s is about 30-50 μM NO_3^-. Although denitrification appears to have responded to eutrophication, the rates observed in this study accounted for only 3-6% of the NO_3^- applied weekly to the water column in the low nutrient pond and 2-4% in the high nutrient pond. Assimilatory uptake was the dominant process causing loss of NO_3^- from the water column in these ponds following fertilization.

Oxygen transport to estuarine sediments can be facilitated by the presence of seagrasses (Oremland and Taylor 1977; Iizumi et al. 1980) which may promote nitrification in otherwise anaerobic environments. This enhancement of NO_3^- production should increase denitrification rates if NO_3^- is the limiting factor. In this study we did not observe significant differences in denitrification rates between vegetated and unvegetated sediments in either the high or low nutrient treatments. Bremner and Blackburn (1979) reported that acetylene inhibits nitrification as well as denitrification in agricultural soils, so that our acetylene-blockage technique may not have been appropriate for detecting such nitrification related effects.

The significantly lower NH_4^+ concentrations in the interstitial waters of vegetated sediments in the low nutrient pond may reflect root uptake by SAV. The average NH_4^+ difference in a m^2 of sediment, 10 cm deep, between vegetated and unvegetated sites was 140 μM. Based on a bulk density of 1.8 g/cm^3 and 20% water content for these sediments described above, this is equivalent to 6300 μmol N. Root biomass in this pond was 20 gdw/m^2 and root uptake was about 4 μg-at N$\cdot$gdw$^{-1}\cdot$ h^{-1} (Stevenson et al., in prep.) resulting in total SAV uptake of 1900 μmol N$\cdot$m$^{-2}\cdot$d^{-1}. This is equivalent to 30%/d of the difference between these two areas and represents a turnover time of 1.4 and 4.7 d of the sediment NH_4^+ pools in the low and high nutrient treatments, respectively.

Algal Response to Nutrient Enrichment

There have been numerous experimental studies of phytoplankton response to nutrient enrichment in microcosms of various sizes and shapes. Generally, these studies have observed rapid response of phytoplankton P_a to fertilization (e.g., Edmondson and Edmonson 1947; Edmondson 1955; Abbott 1967, 1969; Dunstan and Menzel 1971) and elevated but variable algal populations and chlorophyll a concentrations (e.g., O'Brien and de Noyelles 1974; Gamble et al. 1977). Typically, the phytoplankton response is not linear with respect to nutrient additions (O'Brien and de Noyelles 1977; Gamble et al. 1977; Nixon, personal communication), and a variety of factors may be responsible for mollifying or exacerbating this relation. These include nutrient interactions with sediment, grazing on plankton, competition with periphyton and macrophytes, or limitation by carbon or other requisite substances.

Several studies have involved fertilization of experimental systems containing submerged vascular plants, and these have similarly observed increases of phytoplankton biomass at high doses. However, a much reduced response was reported at low and moderate nutrient doses, perhaps, as a result of competition with macrophytes (Mulligan and Baranowski 1969; Mulligan et al. 1976; Hall et al. 1970). In a recent paper Phillips et al. (1978) argued that phytoplankton response was a secondary effect of fertilization, following initial periods of prolific epiphytic growth. Apparently, this increased phytoplankton growth in response to nutrient additions is not confined to microcosms and occurs also in nature, as strong correlations between the two have been observed for lakes (Schindler 1978) and estuaries (Boynton et al. 1982).

Phytoplankton biomass (chlorophyll a) exhibited a strong and very significant increase under high nutrient treatment in our experimental ponds (Fig. 23). However, low and medium treatments seemed to have little effect on plankton biomass. While this comparatively reduced phytoplankton response seen at moderate treatments may be a result of the inability of our sampling scheme to detect ephemeral blooms (with measurements 1-3/wk), it may also indicate an inferior ability of phytoplantkon to compete with SAV and their epiphytes for limited nutrients. We did see increases in chlorophyll a of 1-2 orders of magnitude occurring at high treatment over the course of 1-2 days (Table C-2), suggesting the highly variable nature of these populations. For the most part, when chlorophyll a levels were > 2 μg/ℓ , plankton communities were virtually monospecific, dominated by small coccoid chlorophytes. Undoubtedly, the low mixing rates in these ponds influenced competitive selection conferring advantage to motile and bouyant species. Other investigators have also reported widely fluctuating chlorophyll a levels in response to nutrient additions (Edmondson 1955; O'Brien and deNoyelles 1974) and have attributed this erractic behavior to such factors as pH (carbon limitation) and grazing. Water column attenuation of PAR followed a similar pattern relative to treatment, while total seston exhibited a small but statistically insignificant increase with treatment (Fig. 23). Thus, it appears that living phytoplankton cells generally represented a small fraction of the total seston, but exerted a large effect on PAR attenuation.

Over the last decade an expanding literature has suggested that growth of epiphytic algae in response to fertilization may be more important than phytoplankton relative to effects on submerged vascular plants (Moss 1976; Phillips et al. 1978; Cattaneo and Kalff 1980; Harlin and Thorne-Miller 1981; Sand-Jensen and Søndergaard 1981). Interpretation of these data is complicated by the fact that micro and macro-algae may exhibit differential responses in time (Phillips et al. 1978; Harlin and Thorne-Miller 1981), and there are no previous controlled experiments in brackish or marine environments.

In our study epiphytes exhibited a different response to fertilization than did phytoplankton, where all treatments (low, medium and high) experienced elevated levels of epiphytic material compared to controls. By the final sampling in August, epiphytic material averaged 0.1, 1.9, 2.8 and 2.9 g/gdw (SAV) for control, low, medium and high treatments, respectively (Fig. 13).

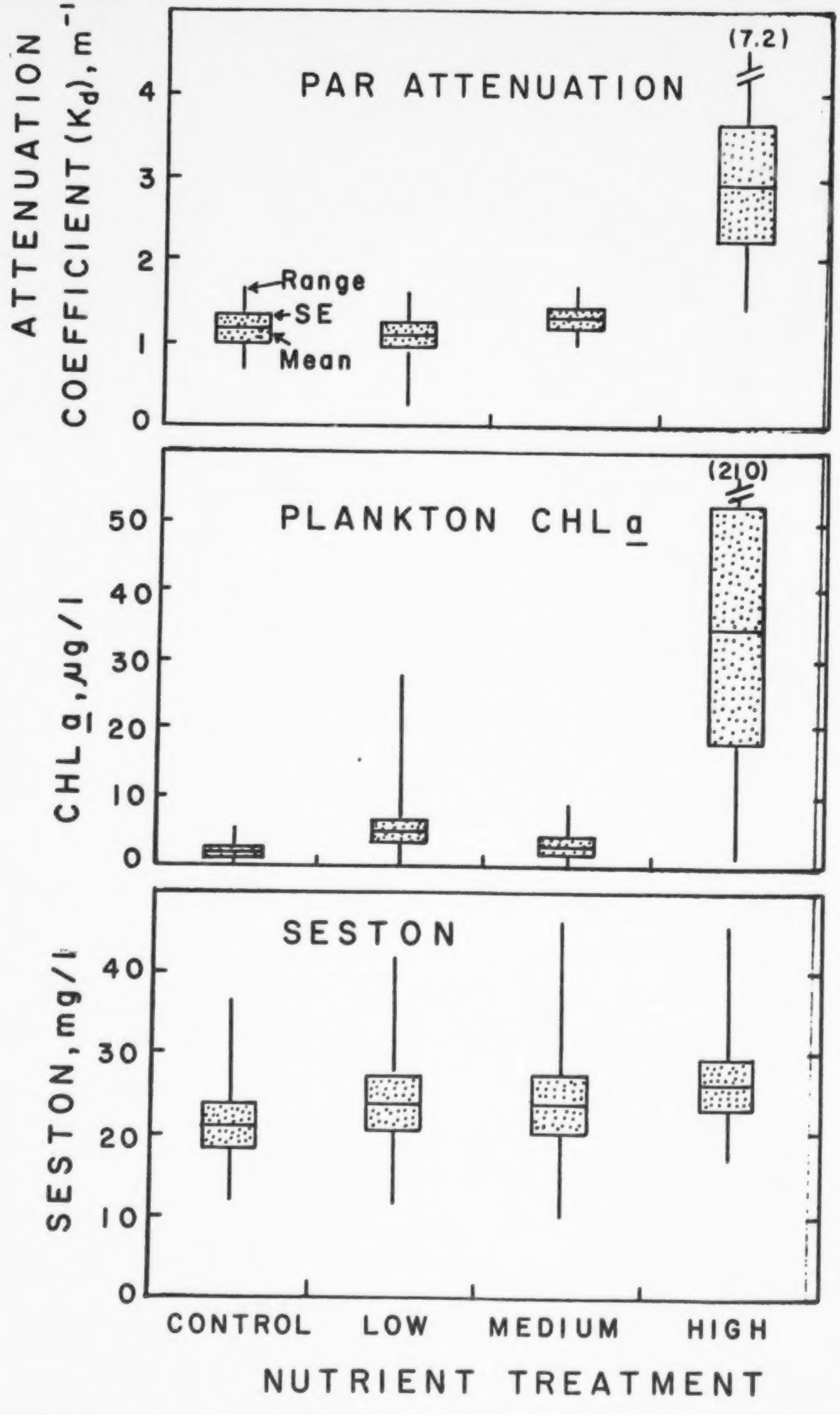

Figure 23. The response of photosynthetic active radiation (attenuation coefficient, K), plankton chlorophyll a (µm/ℓ) and total sestion (mg/ℓ) of the water in pond mesocosms subjected to four nutrient treatments.

The same rank occurred for epiphytic chlorophyll-a which averaged 0.08, 0.37, 0.75 and 1.58 mg/gdw(SAV) in August (Fig. 14). The levels of epiphytic material in nutrient treated ponds are similar to those observed by Staver et al. (1981) for Chesapeake Bay and by Menzie (1979) for Hudson River upper estuary. Control levels are comparable to those observed by Staver et al. (1981) at Parson's Island in July. It appears that in the competition for light and nutrients between phytoplanktonic and epiphytic algae, the latter have an edge under all but the most extreme nutrient additions where phytoplankton are very competitive.

PAR Attenuation by Algal Biomass

An effect of nutrient-stimulated algal growth, which is of importance for the production of submerged vascular plants, is the attenuance of PAR. In our experiment PAR downwelling attenuation coefficients (K) exhibited a moderate but statistically significant correlation ($r^2 = 0.32$) with total seston (Fig. 25A). The slope of this relation (0.0396) is about twice that which we determined experimentally (0.0174) for inorganic sediments (Kemp et al. 1981), suggesting that a greater percentage of the material suspended in the ponds was pigmented (so as to absorb rather than merely reflect efficiently in the 400-700 nm wavelength range). Another indication that higher values of K were associated with fertilized systems is seen in Fig. 24B, where a strong correlation ($r^2 = 0.69$) is obtained between total nitrogen in the water column and PAR attenuance.

Overall, the relation between K and planktonic chlorophyll a was variable, particularly at low pigment levels where other materials dominated the seston (Fig. 25A). However, if we consider only the few instances where algal cells comprised a major fraction of sestion (>10%), a very strong correlation occurs ($r^2 = 0.95$). Here (Fig. 25A) we estimated dry weight of algal cells by assuming a carbon:chlorophyll a ratio of 100 (Hunter and Laws 1981) and a dry weight:carbon ratio of 2.5 (Parsons et al. 1961). The slope of this correlation (0.0146) is remarkably consistent with low input of inorganic sediments (Riley 1956; Scott 1978). These values, however, are about twice those obtained experimentally for pure algal cultures of Dunaliella sp. and Pseudoisochrysis sp. (0.0061 and 0.0081) at our laboratory (Kemp et al. 1981), or those reported elsewhere (e.g., Parsons and Takahashi 1973; Bannister 1974). This discrepancy is likely attributable to the additional presence of considerable amounts of plankton detritus and inorganic solids in pond water columns.

Epiphytic material also reflects and absorbs PAR before reaching SAV leaf surfaces. We obtained an excellent regression between PAR attenuance through a plane and epiphytic biomass ($r^2 = 0.87$) with a slope of 0.36% $(mg/cm^2)^{-1}$. Surprisingly, this is very similar to the relation obtained for seston (with some simple unit conversions, the slope becomes 0.396% $(mg/cm^2)^{-1}$), suggesting that the attenuance properties (per unit wt.) of seston and epiphytes are comparable.

Several previous studies have examined this relationship between epiphytic material and attenuance. Phillips et al. (1978) measured attenuations at several wavelengths for glass slides colonized by algal epiphytes. They provide cell counts as a measure of biomass, and we converted these to mg

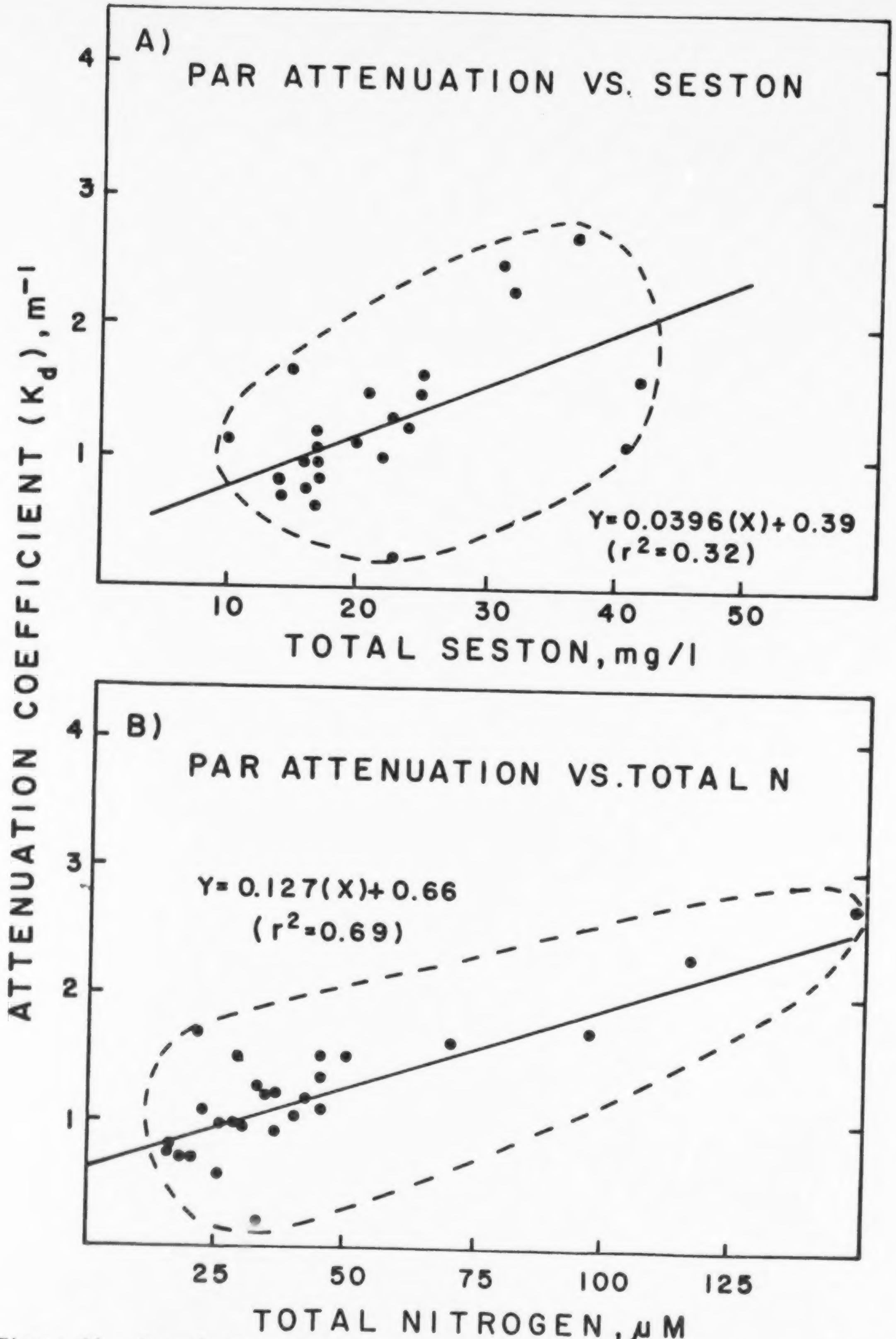

Figure 24. Correlations among the attenuation of photosynhtetic active radiation of the water column in ponds treated with nutrients and A) total seston concentration (mg/ℓ) and B) total nitrogen concentration (μm).

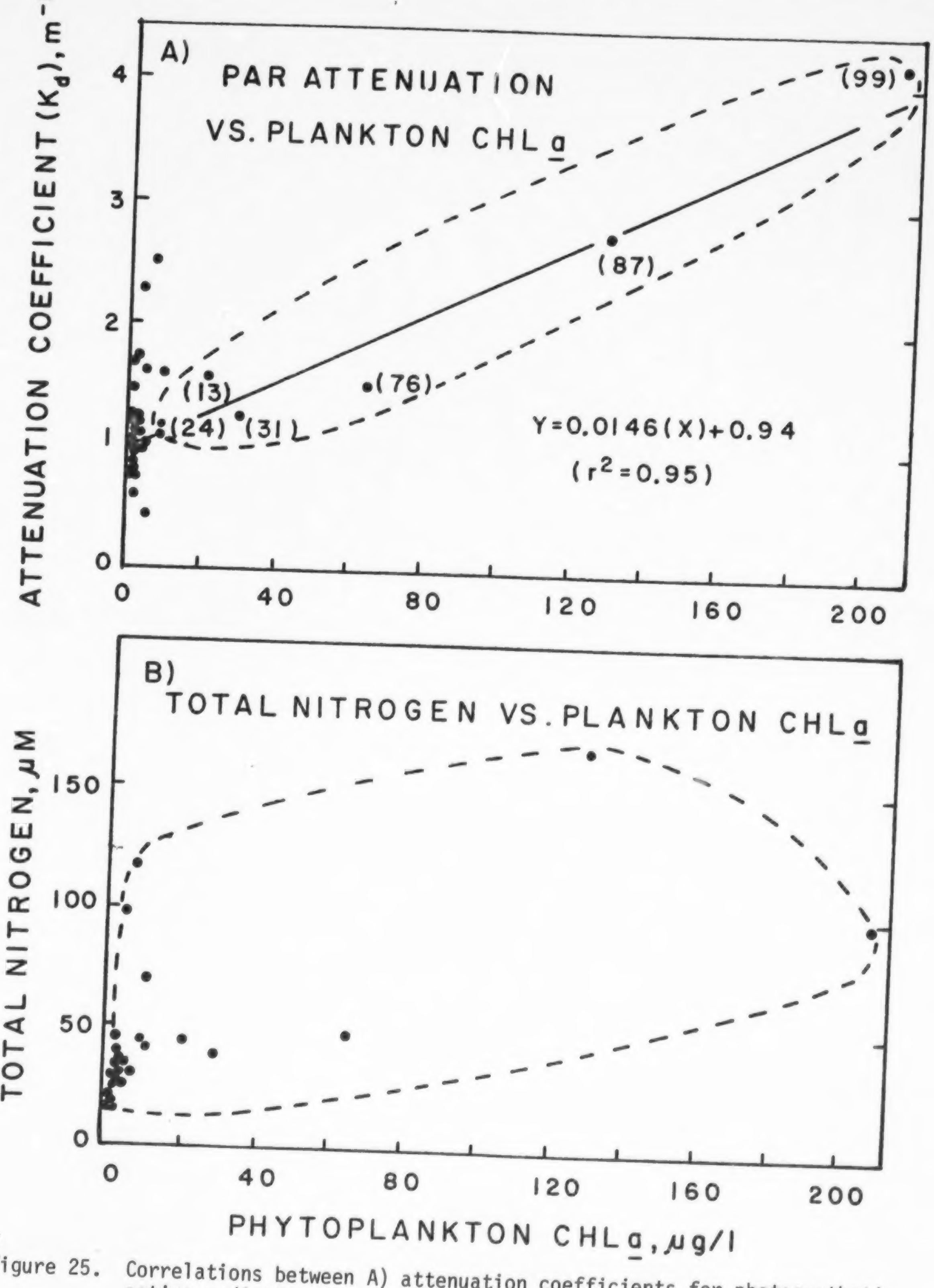

Figure 25. Correlations between A) attenuation coefficients for photosynthetic active radiation and plankton chlorophyll a, and B) total nitrogen concentrations (μm) and plankton chlorophyll a in water column of ponds treated with nutrients.

dry wt. using chl *a*/cell data of Hickman (1971) from a nearby locale and dry wt/chl *a* data of Staver et al. (1981). The resulting relationship for 3 levels of epiphyte colonization is almost identical with ours, where 10 mg/cm^2 results in about 84% absorption and 2 mg/cm^2 causes 52% absorption at 615 nm. On the other hand, the data of Borum and Wium-Andersen (1980) indicate a less pronounced effect, so that 12 mg/cm^2 results in only 56% reduction in PAR. Presumably, the epiphytic material in the latter study contained a greater percentage of nonabsorbing inorganic particulates. Finally, the extensive relationships reported by Sand-Jensen and Søndergaard (1981) for 4 Danish lakes appear to be very similar to those observed in the present study. Unfortunately, each study has reported the results in different units, so that these comparisons must be considered tentative.

Epiphyte Effects on SAV

The development of epiphytic communities on SAV leaves can reduce vascular plant production through several mechanisms other than PAR attenuation, including the reduction of diffusive transport of N, P and CO_2. Furthermore, the relationship between light reduction and loss of photosynthesis is nonlinear except at PAR levels below I_k (intersection of initial slope of photosynthesis-irradiance curve with maximum photosynthesis value). Thus, it is important to examine directly the effects of epiphytic material on SAV photosynthesis.

We tested this relationship between epiphytes and SAV P_a twice, once in July using O_2 production as a measure of P_a, and once in September using ^{14}C incorporation. Significant relationships between reduction in P_a and epiphyte biomass were obtained (Fig. 26) in both cases (r^2 = 0.84 and 0.69 for O_2 and ^{14}C, respectively). However, the slopes were markedly different, being three times greater in the earlier study. This may be the result of different epiphytic communities during the two experiments, where the September epiphytes were less detrimental per unit dry weight. These experiments were conducted using natural light incubations, and the slightly higher ambient PAR in September could partially explain this difference in slopes. We can compare Figs. 9 and 27 by utilizing the relationship between leaf area and plant dry wt. for *P. perfoliatus* in these ponds (4.7 x 10^3 cm^2/g, Fig. 27). If there was a *one-to-one relation* between light and SAV P_a we would expect about a 90% loss of P_a associated with an increase from 1.0 to 3.0 g epiphyte/g SAV. In fact, the September experiment predicts a 27% reduction in P_a, while the July data indicate a 67% loss of P_a. Thus, the relation to light was not linear for either experiment.

Other investigators have considered the effects of epiphytic colonization on seagrass P_a, but only at a single level of epiphytes. Sand-Jensen (1977) showed that from darkness to saturating PAR levels epiphyte colonized *Zostera marina* exhibited lower P_a than clean plants. The % reduction was greatest however (ca. 50%) through the initial linear portion of the curve. He reported inhibition due to epiphytes even at PAR of about 1200 μEin $m^{-2}s^{-1}$. Yet, even with an 80% reduction of incident PAR, remaining light would be near saturation. Sand-Jensen (1977) also showed a reduction in CO_2 uptake by *Z. marina* due to epiphytes except at the highest concentrations of inorganic carbon, presumably capable of saturating plant demand. Penhale and Smith (1977)

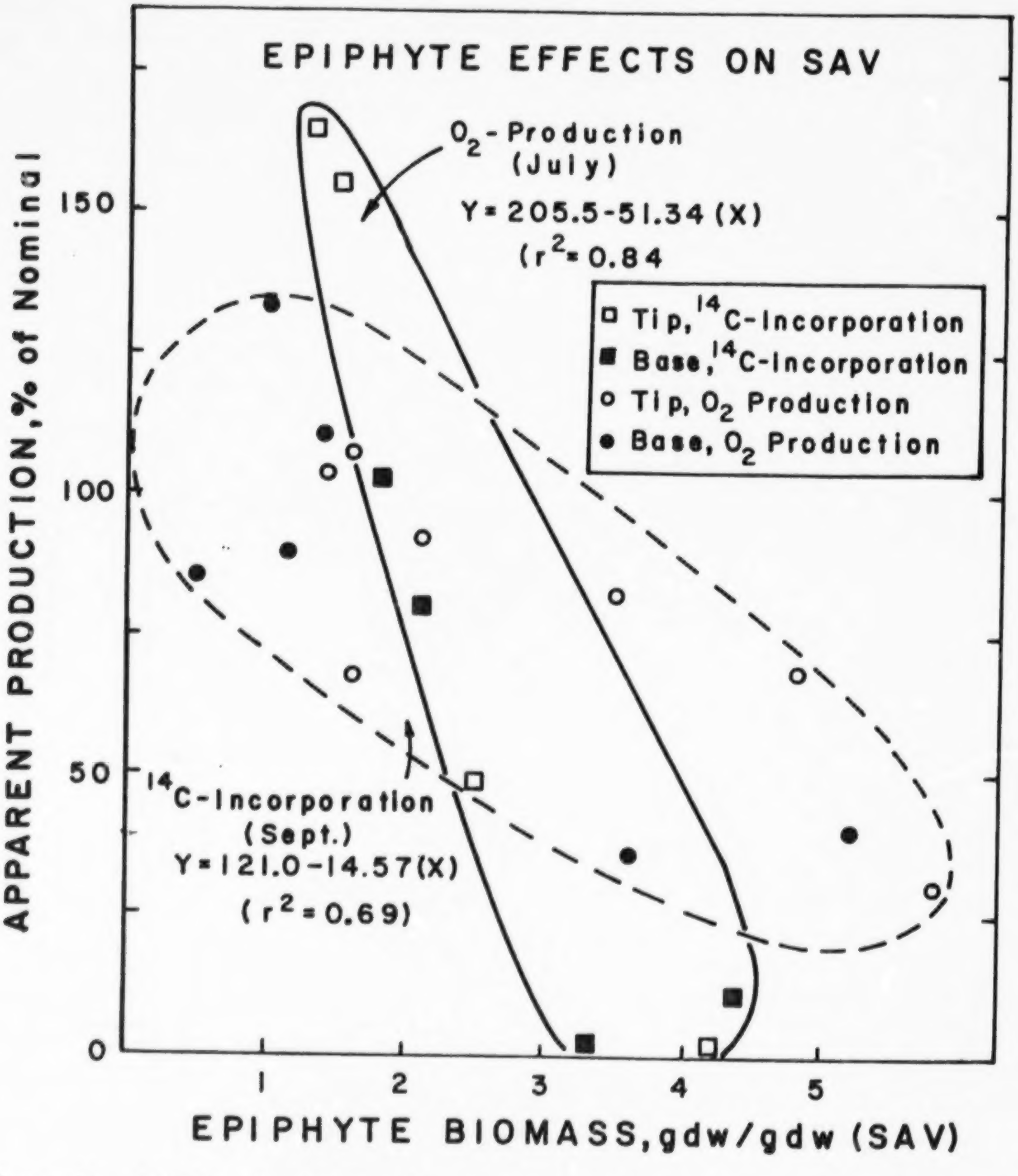

Figure 26. Effects of epiphyte biomass [gdw/gdw(SAV)] on the normal apparent production of Potamogeton perfoliatus (% of production of plants cleared of epiphytic material) based on experiments using ^{14}C-incorporation and dissolved oxygen evolution to measure plant metabolism.

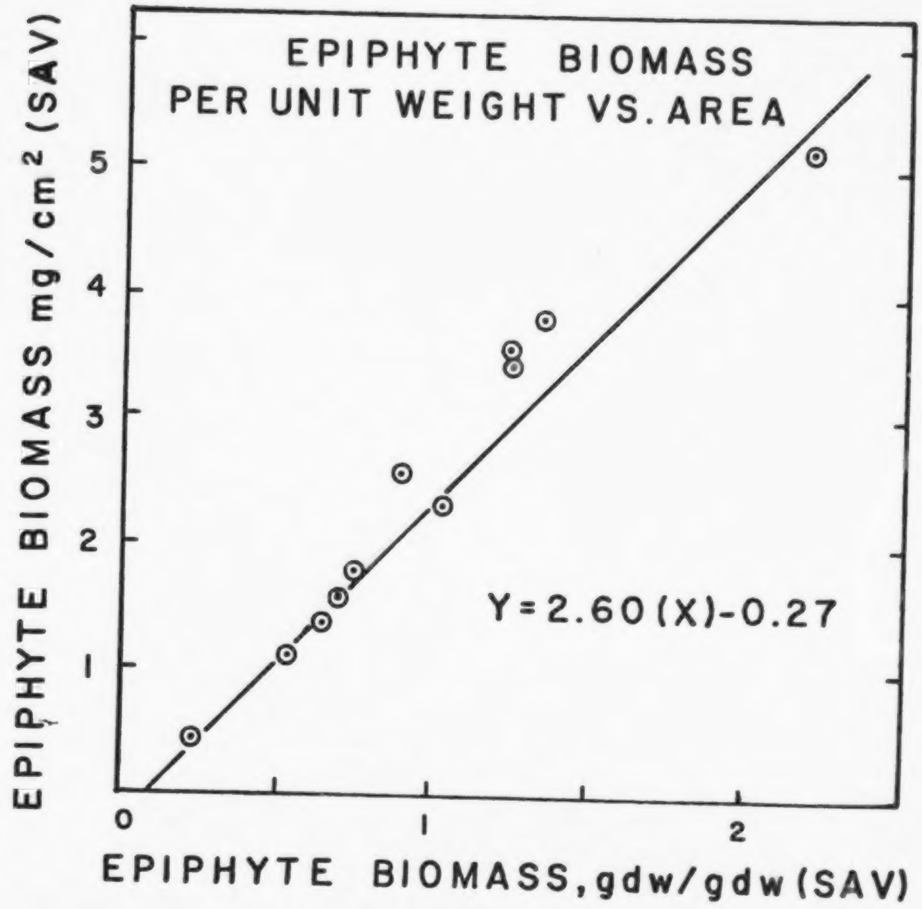

Figure 27. Effects of epiphyte biomass on Potamogeton perfoliatus expressed as area (mg/cm^2) and weight [gdw/gdw(SAV)].

conducted similar experiments, but found little epiphyte effect except above 800 µEin m^{-2}s^{-1}, suggesting that the reduction of diffusive uptake of minerals (where demand was greatest at high PAR) may have been more important than light attenuation.

In a recent paper, Sand-Jensen and Søndergaard (1981) have argued that light attenuation associated with epiphytic growth exerts a far greater impact of SAV than that due to phytoplankton in Danish lakes subjected to different degrees of fertilization. This is probably the case for these lakes where phytoplankton pigments are < 10 µg/ℓ and watercolumn attenuation coefficients are generally < 1.0/m. In the shallow waters of upper Chesapeake Bay, however, attenuation coefficients of 1-4/m are not uncommon, and chl *a* typically ranges from 5-30 µg/ℓ (Boynton et al. 1981). Most of the attenuation here is probably due to suspended sediments, although plankton are important.

In our nutrient enrichment experiments, both plankton and epiphytes contributed substantially to PAR attenuation, particularly in the high nutrient dosed ponds. For example, with a typical summer PAR of 1200 µEin m^{-2}s^{-1} incident at the water surface and a water column $K = 3.0$/m, there would be 60, 270 and 570 µEin m^{-2}s^{-1} available at the sediment surface, at 0.5 m, and at 0.25 m depth. Typical values of compensation light levels (I_c) are about 50-75 µEin m^{-2}s^{-1}, so that net production is not possible at the sediment surface. Given a typical value of epiphytic biomass of 4.0 gdw/gdw (SAV), we would find 30 and 10 µEin m^{-2}s^{-1} available for leaves at 0.25 m and 0.5 m, respectively, below the water surface. Thus, unless these vascular plants can adapt by pigment and enzymatic shifts to such rigorous light regimes, they cannot exist under the combined stress of turbidity plus epiphytic material.

SAV Biomass Response to Nutrient Enrichment

Nutrient loading rates ≥ 60 µM N/wk and 6 µM P/wk during the growing season caused a significant decline in SAV biomass in our pond mesocosms. Negative biomass responses were not observed until 60 days following the initial fertilization and can be attributed to (among other things) decreased light availability in response to increased phytoplankton and epiphyte growth. The results of this study agree with observations of other fertilization experiments using ponds, and together these studies show the response of SAV to a gradient of N and P loading rates (Fig. 28). All of these studies used 710 d fetilization frequencies for periods ranging from 6-23 wk. Our study is the only nutrient enrichment experiment reported here on estuarine plant communities.

Loading rates up to 30 µM N/wk and 3.0 µM P/wk above ambient nutrient conditions resulted in no apparent effect on SAV biomass (Fig. 28). At 50 µM N/wk and 2.2 µM P/wk Moss (1976) observed lower biomass levels compared to low nutrient and control treatments. However, at slightly higher N and P loading rates, Howard-Williams (1981) showed no adverse effects of nutrients to *Potamogeton pectinatus*. Above 60 µM N/wk and 6 µM P/wk, SAV consistently exhibited declines in biomass compared to control treatments (Fig. 28). At 300 µM N/wk and 16 µM P/wk, SAV biomass was completely eliminated in 5 of 6 experimental ponds (Mulligan et al. 1976). Investigators have attributed differences observed among these studies to species tolerance to nutrient enrichment (Mulligan and Baranowski 1969, Ryan et al. 1972, Moss 1976). A

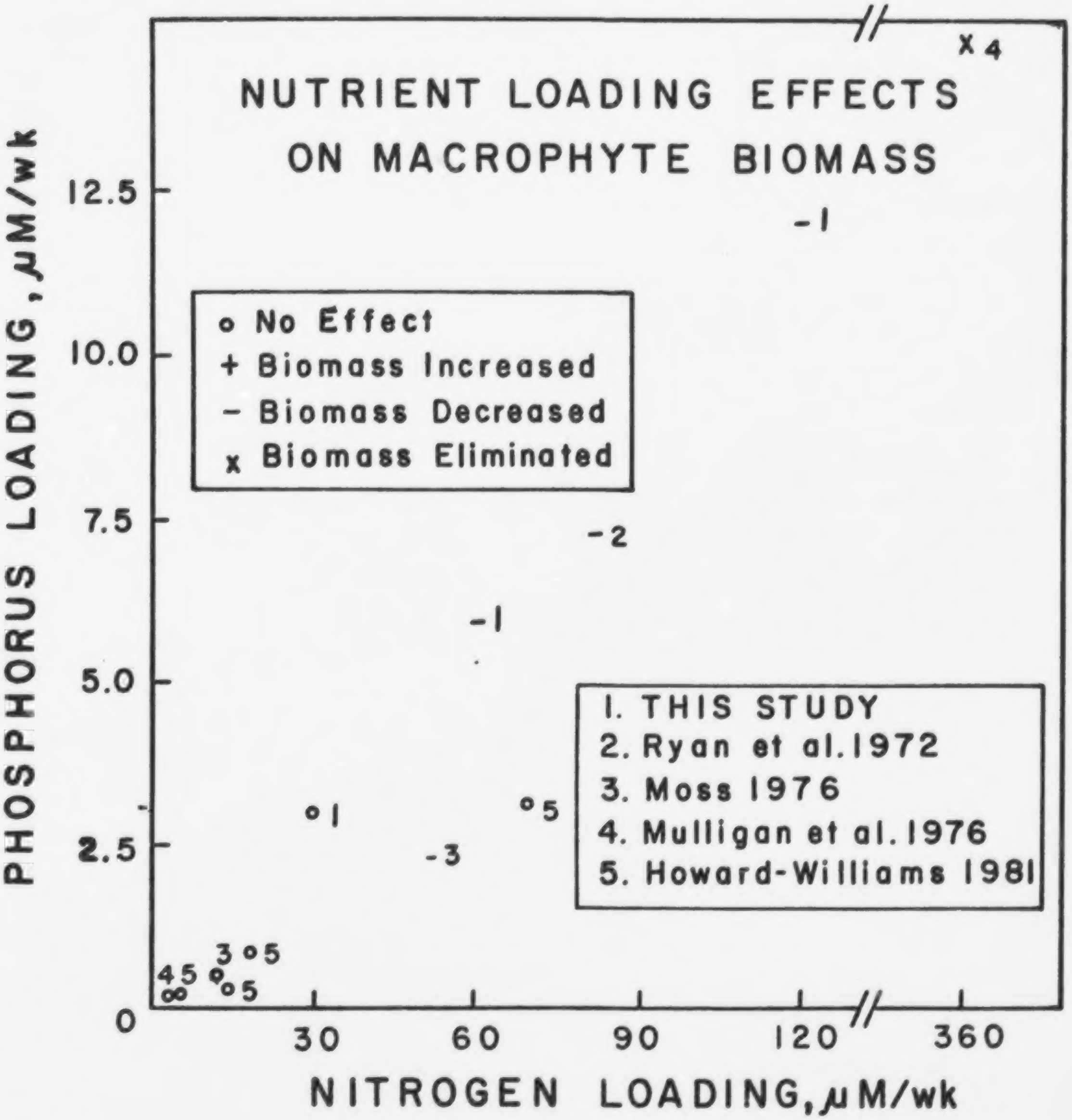

Figure 28. The response of submersed aquatic vegetation in experimental ponds to various loading rates of nitrogen and phosphorus.

range of factors may influence these apparent taxonomic differences; including nutrient absorption capacity, adaptations to shading, and high initial biomass conditions which may have ameliorated nutrient inputs (Howard-Williams 1981). Thus, all of these studies show remarkable similarity in the response of SAV communities to increased nutrient loadings.

Response of Community Metabolism to Fertilization

Nutrient enrichment of pond ecosystems is a standard practice for increasing fish yields by stimulating primary production. In this study, medium nutrient enrichments of 60 µM N/wk and 6 µM P/wk increased net community production over controls; however at doubled fertilization rates net community production declined. Hephner (1962) observed a similar response of fertilized ponds in which modest nutrient additions resulted in a total production rate of 462.0 mg C•m-2•h-1 compared to 384.5 mg C•m-2•h-1 at higher fertilization. Although surface photosynthesis were greater at high nutrients, productivity at lower depths was inhibited from shading by surface plankton. In our study, high nutrient enrichmnet resulted in a decline in SAV biomass and a subsequent reduction of total community net production. This decrease in SAV corresponded to increases in both phytoplankton and epiphytes. However, since total community production decreased at high dosage, the increased phytoplankton and epiphytic growth was insufficient to compensate for the loss of vascular plants.

The role of SAV beds as habitat and indirect food sources for many sport and commercial fisheries has been well established for Chesapeake Bay (Boynton 1982). The relative balance between production and respiration (P:R) for these SAV communities provides an index of their ability to fill this role. In our study, low nutrient levels resulted in increased P:R ratios from 1.8-2.8; however, as nutrient levels increased, community P:R ratio decreased to <1.0 (Fig. 29). Under these eutrophic conditions, SAV communities no longer contribute net energy to estuarine food webs . The response of these experimental estuarine systems to elevated nutrient levels followed the general push-pull patterns discussed by Odum et al. (1979) for ecosystems under stress.

SAV Strategy for Growth and Production

Net photosynthesis (P_a) of *Potamogeton* and *Ruppia* were distinctly different in both vertical (along length of the plant) and diurnal patterns. These diurnal trends suggest that *P. perfoliatus* is more responsive to light changes than is *R. maritima*. Nonetheless, the total apparent production of both plants was about 25 mg O_2•gdw^{-1}•d^{-1}. Respiration was relatively constant throughout the day for both plants, indicating that production and respiration may be coupled more closely in *R. maritima*.

Maximum P_a along the length of *Potamogeton* was observed in the tip of the plant, whereas P_a in *Ruppia* was nearly constant along its entire length (Fig. 16). A gradient of epiphyte biomass with lower concentrations at the tip has been observed in the field for mature *Potamogeton* (Staver et al. 1981) and corroborated with data from the ponds. These gradients suggest that under ambient conditions, the tip of *Potamogeton* must be paramount to the

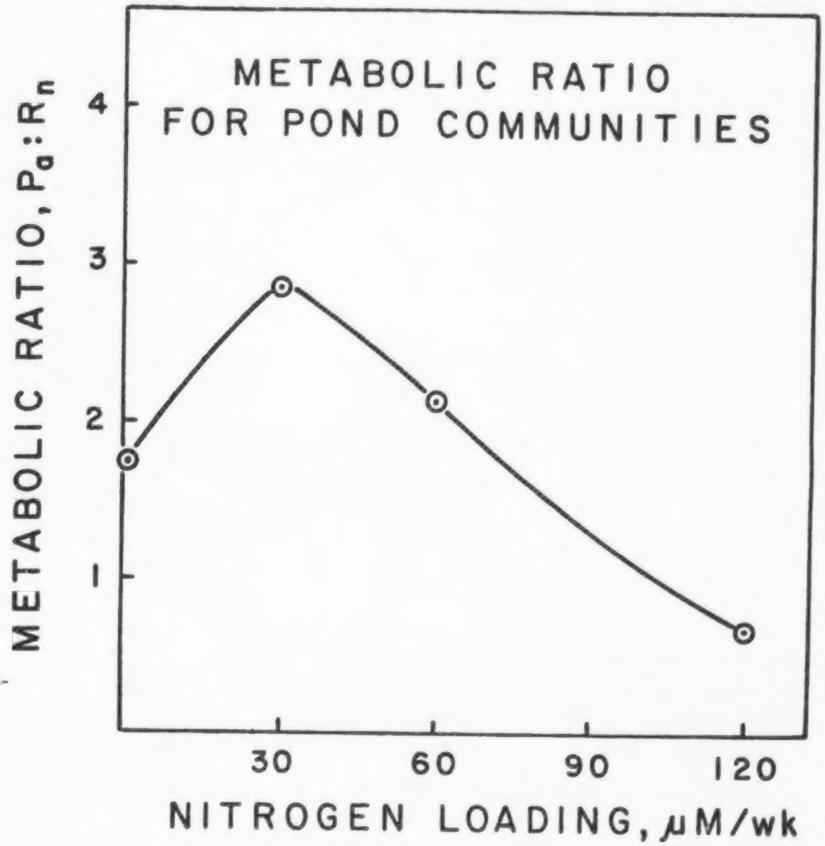

Figure 29. The apparent production to nighttime respiration ratio of ponds at four nutrient treatments.

energy economy of the entire plant, particularly when demands for belowground production and flowering are included. When this terminal section of *Potamogeton* is shaded either by epiphytic or suspended material, the balance between production and consumption of energy becomes negative as observed experimentally in this study (Fig. 17). As the temporally and spatially integrated energy balance for these vascular plants approaches zero, a shift in reproductive strategy may become evident.

Potamogeton perfoliatus reproduces sexually by means of seed production during the summer, and asexually by formation of buds on the apex of rhizomes at the growing season end. As observed for *P. crispus*, the advent of a new growing season for this genus seems to be dominated by asexually produced propagules rather than seeds (Rogers and Breen 1980). The starch content of these structures enables plants to grow under adverse environments which may exist early in the growing season (Rogers and Breen 1980). Fixed carbon must be translocated from sites of production to storage for this propagation scheme to be successfull (Harrison 1978). Under high nutrient conditions, the shading of the *Potamogeton* shoot tips may decrease the net energy available for bud formation. In a survey of belowground vegetation in our experimental ponds during November, buds were found in all ponds except one of those receiving high nutrient treatment, which had no belowground vegetation. During the subsequent growing season, no vascular plants were observed in this pond. Further studies are needed to investigate factors affecting net production in these plants, particularly as related to respiration patterns, reproductive structures and germination success.

SUMMARY

Eight experimental ponds containing submerged aquatic vegetation (SAV) were subjected in duplicate to 4 levels (including control) of fertilization from June to August 1981. Nutrients were added on a weekly ccycle with highest loadings corresponding to those typical for brackish environments in the upper Chesapeake Bay receiving agricultural runoff. Nutrients were removed to ambient levels within 1-3 d following treatment except in the high dose systems after the fourth application, when NO_3^- levels persisted well above controls for the entire period. Vascular plant tissue nutrients responded to treatment with about 3% N at high dosage and 1.2% for controls. It appears that sediment denitrification also followed treatment level; however, there was no evidence for any influence of SAV on this process. Seston and, in particular, phytoplanton chlorophyll *a* increased with fertilization, and pronounced blooms were evident under high dosage. Of the total seston, phytoplankton exerted the greatest influence on attenuation of photoysnthetically active radiation (PAR), so that there was insufficient light for SAV growth at the sediment surface during blooms. An extensive epiphytic community developed on plants in all nutrienttreted ponds at densities similar to those observed in nature on senescent plants. Attenuation of PAR by these epiphytes was considerable, with >90% absorption and reflection of incident radiation resulting from typical epiphytic densities in treated systems. However, without any PAR attenuance in the overlying water, such epiphytic growth would be insufficient to reduce light below photosynthetic compensation levels, and net SAV growth could occur. Thus, PAR attenuation by both seston and epiphytes is necessary to effect

plant mortality. In fact, SAV biomass decreased significantly in high treatment compared to controls within 60 d following initiation of the experiment. Moreover, the integrated primary production of these entire pond communities was significantly reduced with the loss of SAV, eventhough phytoplankton and epiphytic growth were enhanced. This premature loss of SAV may only amount to a shortening of the growing season rather than a population decimation unless plant reproduction is also detrimentally affected. Some preliminary evidence suggests that the inability of these stressed plants to maintain a balance between photosynthesis and respiration may prevent sufficient translocation of energy to below-ground overwintering structures. It is hypothesized that without this vegetative energy storage, spring budding may not be possible.

LITERATURE CITED

Abbott, W. 1969. Nutrient studies in hyperfertilized estuarine ecosystems. I. Phosphorus studies. In: S.H. Jenkins (ed.), Advances in water pollution research. Pergamon Press, New York.

Abbott, W. 1967. Microcosm studies on estuarine waters. II. The effects of single doses of nitrate and phosphate. J.W.P.C.F. 39:113-122.

Adams, Franklin, S., Herbert Cole, Jr., and Lowny B. Massie. 1973. Element constitution of selected aquatic vascular plants from Pennsylvania: submersed and floating leaved species and rooted emergent species. Environ. Pollut. 5:117-147.

Adams, Franklin S., David R. MacKenzie, Herbert Cole, Jr., and Marilyn W. Price. 1971. The influence of nutrient pollution levels upon element constitution and morphology of Elodea canadensis Lich. in Michx. Enviorn. Pollut. 1:285-298.

Bannister, T.T. 1974. A general theory of steady state phytoplankton growth in a nutrient saturated mixed layer. Limnol. Oceanogr. 19:13-30.

Bayley, S., V.D. Stotts, P.F. Springer, and J. Steenis. 1978. Changes in submerged aquatic macrophyte populations at the head of Chesapeake Bay, 1958-1975. Estuaries 1:73-84.

Best, E.P.H. 1977. Seasonal changes in mineral and organic components of Ceratophyllum demersum and Elodea canadensis. Aquatic Bot. 3:337-348.

Best, E.P.H. 1980. Effects of nitrogen on the growth and nitrogenous compounds of Ceratophyllum demersum. Aquatic Bot. 8:197-206.

Billen, G. 1978. A budget of nitrogen recycling in North Sea sediments off the Belgian coast. Estuarine Coastal Mar. Sci. 7:127-146.

Borum, J. and S. Wium-Anderson. 1980. Biomass and production of epiphytes on eelgrass (Zostera marina L.) in the Øresund, Denmark. Ophelia (Suppl.) 1:57-64.

Boyd, C.E. 1971. The limnological role of aquatic macrophytes and their relationship to reservoir management. Res. Fish. and Limnol. 8:153-166.

Boyd, C.E. 1970. Vascular aquatic plants for mineral nutrient removal from polluted waters, Economic Botany, Vol. 24:95-103.

Boyd, C.E. and E. Scarsbrook. 1975. Influence of nutrient additions and initial density of plants on production of waterhyacinth Eichhornia crassipes. Aquatic Botany 1:253-261.

Boynton, W.R. and K.L. Heck. 1981. Ecological role and value of submerged macrophyte communities: A scientific summary. Univ. of Md. Center for Environmental and Estuarine Studies, Box 38, Solomons, Md. Ref. No. 81-194CBL.

Boynton, W.R., W.M. Kemp and C.W. Keefe. 1982. A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production, pp. 69-90. In: V.S. Kennedy (ed.), Estuarine Comparisons, Academic Press, N.Y.

Boynton, W.R. et al. 1981. Influence of submerged macrophyte communities on turbidity and sedimentation in littoral zones of northern Chesapeake Bay, pp. 842-878. In: W.M. Kemp, W.R. Boynton, J.C. Stevenson and J.C. Means (eds.), Submerged aquatic vegetation in Chesapeake Bay. Univ. of Md., Center for Environmental and Estuarine Studies, Contribution No. 81-28-HPEL.

Bremner, J.M. and A.M. Blackmer. 1979. Effects of acetylene and soil water content on emission of nitrous oxide from soils. Nature 280:380-381.

Cambridge, M.L. 1979. Cockburn Sound study technical report on seagrass. Dept. Conservation and Environment, Western Australia. Rep. No. 7.

Cattaneo, A. and J. Kalff. 1980. The relative contribution of aquatic macrophytes and their epiphytes to the production of macrophyte beds. Limnol. Oceanogr. 25:280-289.

Cattaneo, A. and J. Kalff. 1979. Primary production of algae growing on natural and artificial aquatic plants: A study of interactions between epiphytes and their substrate. Limnol. Oceanogr. 24:1031-1037.

Copeland, B.J. and W.R. Duffer. 1964. Use of a clear plastic dome to measure gaseous diffusion rates in natural waters. Limnol. Oceanogr. 9:494-499.

Dale, H.M. and G.E. Miller. 1978. Changes in the aquatic macrophyte flora of Whitewater Lake near Sudbury, Ontario from 1947 to 1977. Canad. Field-Naturalist 92:264-270.

Den Hartog, C. and P.J.G. Polderman. 1975. Changes in seagrasses populations of the Dutch Waddenzee. Aquatic Botany 1:141-147.

Dunstan, W.M. and D.W. Menzel. 1971. Continuous cultures of natural populations of phytoplankton in dilute, treated sewage effluent. Limnol. Oceanogr. 16:623-632.

Edmondson, W.T. and Y.H. Edmondson. 1947. Measurements of production in fertilized salt water. J. Mar. Res. 6:228-245.

Edmondson, W.T. 1955. Factors affecting productivity in fertilized salt water. Deep-Sea Res. 3:451-464.

Fitzgerald, George P. 1969. Field and laboratory evaluations of bioassays for nitrogen and phosphorus with algae and aquatic weeds. Limnol. and Oceanogr. 206-212.

Gamble, J.C., J.M. Davies, and J.H. Steele. 1977. Loch Ewe bag experiment, 1974. Bulletin of Marine Science 27:146-175.

Gerloff, G.C. and P.H. Krombholz. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperms aquatic plants. Limnol. Oceanogr. 11:529-537.

Gossett, D.R. and W.E. Norris, Jr. 1971. Relationship between nutrient availability and content of nitrogen and phosphorus in tissues of the aquatic macrophyte, Eichornia crassipes (Mart.) Solms. Hydrobiologia, 38(1):15-28.

Hall, D.J., W.E. Cooper, and E.E. Werner. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnol. Oceanogr. 15:839-928.

Harlin, M.M. and B. Thorne-Miller. 1981. Nutrient enrichment of seagrass beds in a Rhode Island coastal lagoon. Marine Biology 65:221-229.

Harrison, P.G. 1978. Patterns of uptake and translocation of ^{14}C by Zostera americana den Hartog in the laboratory. Aquatic Botany 5:93-97.

Heinle, D.R., C.F. D'Elia, J.L. Taft, J.S. Wilson, M. Cole-Jones, A.B. Caplins, and L.E. Cronin. 1980. Historical review of water quality and climatic data from Chesapeake Bay with emphasis on effects of enrichment. Univ. MD Center for Environ. Estuarine Studies. Ref. No. 80-15-CBL, Solomons, MD.

Henriksen, K., J.I. Hansen and T.H. Blackburn. 1981. Rates of nitrification, distribution of nitrifying bacteria, and nitrate fluxes in different types of sediment from Danish waters. Marine Biology 61:299-304.

Hepher, B. 1962. Primary production in fishponds and its application to fertilization experiments. Limnology and Oceanography 7:131-136.

Hickman, M. 1971. The standing crop and primary productivity of the epiphyton attached to Equisetum fluviatile L. in priddy pool. North Somerset. Br. Phycol. J. 6:51-59.

Hiscox, J.D. and G.F. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57:1332-1334.

Howard-Williams, C. 1981. Studies on the ability of a *Potamogeton pectinatus* community to remove dissolved nitrogen and phosphorus compounds from lake water. Journal of Applied Ecology 18:619-637.

Hunter, B.L. and E.A. Laws. 1981. ATP and chlorophyll *a* as estimators of phytoplankton carbon biomass. Limnol. Oceanogr. 26:944-956.

Iizumi, H. and A. Hattori. 1982. Growth and organic production of eelgrass (*Zostera marina* L.) in temperate waters of the Pacific Coast of Japan. III. The kinetics of nitrogen uptake. Aquatic Botany 12:245-256.

Iizumi, H., A. Hattori, and C.P. McRoy. 1980. Nitrate and nitrite in interestitial waters of eelgrass beds in relation to the rhizosphere. J. Exp. Mar. Biol. Ecol. 47:191-201.

Jupp, B.P. and D.H.N. Spence. 1977. Limitations of macrophytes in a eutrophic lake, Loch Leven. II. Wave action, sediments, and waterfowl grazing. Journal of Ecology 65:431-446.

Jupp, B.P., and D.H.N. Spence. 1977. Limitations on macrophytes in a eutrophic lake, Loch Leven, I. Effects of phytoplankton. Journal of Ecology 65:175-186.

Jupp, B.P., and D.H.N. Spence. 1977. Limitations on macrophytes in a eutrophic lake, Loch Leven, I. Effects of phytoplankton. Journal of Ecology 65:175-186.

Kaumeyer, K. et al. 1981. Nutrient dynamics of submerged macrophyte communities in northern Chesapeake Bay, pp. 685-802. *In*: W.M. Kemp, W.R. Boynton, J.C. Stevenson and J.C. Means (eds.), Submerged aquatic vegetation in Chesapeake Bay. Univ. of Md., Center for Environmental and Estuarine Studies, Contribution No. 81-28-HPEL.

Kemp, W.M. et al. 1981. Experimental observations of turbidity/light relations and their influence on submerged macrophytes in northern Chesapeake Bay, pp. 2-29. *In*: W.M. Kemp, W.R. Boynton, J.C. Stevenson, J.C. Means (eds.), Submerged aquatic vegetation in Chesapeake Bay. Univ. of Md., Center for Environmental and Estuarine Studies, Contribution No. 81-28-HPEL.

Koike, I. and A. Hattori. 1979. Estimates of denitrification in sediments of the Bering Sea shelf. Deep-Sea Research 26:409-415.

Lewis, M.R., W.M. Kemp, J.J. Cunningham, and J.C. Stevenson. 1982. A rapid technique for preparation of aquatic macrophyte samples for measuring ^{14}C incorporation. Aquatic Botany 13:203-207.

Lind, C.T. andd G. Cottam. 1969. The submersed aquatics of University Bay: A study in eutrophication. Amer. Midl. Nat. 81:353-369.

McCarthy, J.J., W.R. Taylor, and J.L. Taft. 1977. Nitrogenous nutrition of the plankton in the Chesapeake Bay. I. Nutrient availability and phytoplankton preferences. Limnol. Oceanogr. 22:996-1011.

McNabb, C.D. 1976. The potential of submersed vascular plants for reclamation of wastewater. *In*: J. Tourbier and R.V. Pearson (eds.), Biological Control of Water Pollution. The University Press, Philadelphia.

Menzie, C.A. 1979. Growth of the aquatic plant *Myriophyllum spicatum* in a littoral area of the Hudson River estuary. Aquat. Bot. 6:365-375.

Mihursky, J.A. and W.R. Boynton. 1979. Review of the Patuxent Estuary data base. Chesapeake Biological Laboratory, Solomons, Maryland. University of Maryland, Center for Environmental and Estuarine Studies. Ref. No. 78-157-CBL.

Moss, B. 1976. The effects of fertilization and fish on community structure and biomass of aquatic macrophytes and epiphytic algal populations: an ecosystem experiment. Journal of Ecology 64:313:342.

Mulligan, H.F. and A. Baranowski. 1969. Growth of phytoplankton and vascular aquatic plants at different nutrient levels. Verh. Internat. Verein. Limnol. 17:802-810.

Mulligan, H.F., A. Baranowski and R. Johnson. 1976. Nitrogen and phosphorus fertilization of aquatic vascular plants and algae in replicated ponds. I. Initial response to fertilization. Hydrobiologia 48:109-116.

Nichols, D.S. and D.R. Keeney. 1976. Nitrogen nutrition of *Myriophyllum spicatum*: uptake and translocation of ^{15}N by shoots and roots. Freshwater Biol. 6:145-154.

Nishio, T., I. Koike, and A. Hattori. 1982. Denitrificationn, nitrate reduction, and oxygen consumption in coastal and estuarine sediments. Applied and Environmental Microbiology 43:648-653.

O'Brien, W.J. and F. DeNoyelles, Jr. 1974. Relationship between nutrient concentration, phytoplankton density, and zooplankton density in nutrient enriched experimental ponds. Hydrobiologia 44:105-125.

Odum, E.P., J.T. Finn, and E.H. Franz. 1979. Perturbation theory and the subsidy-stress gradient. Bioscience 29:349-352.

Odum, H.T. and C.M. Hoskin. 1958. Comparative studies on the metabolism of marine waters. Publ. Inst. Mar. Sci. Univ. Tex. 6:159-170.

Oremland, R.S. and B.F. Taylor. 1977. Diurnal fluctuations of O_2, N_2 and CH_4 in the rhizosphere of *Thallassia testudinum*. Limnology and Oceanography 22:566-570.

Oren, A. and T.H. Blackburn. 1979. Estimation of sediment denitrification rates at *in situ* nitrate concentrations. Applied and Environmental Microbiology 37:174-176.

Orth, R.J. 1977. Effect of nutrient enrichment on growth of the eelgrass *Zostera marina* in the Chesapeake Bay, Virginia, USA. Marine Biology 44:187-194.

Orth, R.J. and K.A. Moore. 1981. Submerged aquatic vegetation of the Chesapeake Bay: Past, present and future. Trans. 46th North American Wildlife and Nat. Res. Conference. Wildlife Management Institute, Washington, D.C.

Parsons, T. and M. Takahashi. 1973. Biological oceanographic processes. Pergamon Press, New York.

Parsons, T.R., K. Stephens, and J.D.H. Strickland. 1961. On the chemical composition of eleven species of marine phytoplankters. J. Fish. Res. Bd. Canada 18:1001-1016.

Penhale, P. 1977. Macrophyte-epiphyte biomas and productivity in an eelgrass (Zostera marina) community. J. Exp. Mar. Biol. Ecol. 26:211-224.

Penhale, P.A. and W.O. Smith. 1977. Excretion of dissolved organic carbon by eelgrass (Zostera marina) and its epiphytes. Limnol. Oceanogr. 22:400-407.

Peres, J.M. and J. Picard. 1975. Causes de la rarefaction et de la disparition des herbiers de Posidonia oceanica sur les cotes Francaises de la Mediterranee. Aquatic Botany 1:133-139.

Phillips, G.L., D. Eminson, and B. Moss. 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquatic Botany 4:103-126.

Rogers, K.H. and C.M. Breen. 1980. Growth and reproduction of Potamogeton crispus in a south African lake. Journal of Ecology 68:561-571.

Ryan, J.B., D.N. Riemer, and S.J. Toth. 1972. Effects of fertilization on aquatic plants, water, and bottom sediments. Weed Sci. 20:482-486.

Sand-Jensen, K. 1977. Effect of epiphytes on eelgrass photosynthesis. Aquatic Botany 3:55-63.

Sand-Jensen, K. and M. Sondergaard. 1981. Phytoplankton epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. Int. Revue ges. Hydrobiol 66:529-552.

Schindler, D.W. 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnol. Oceanogr. 23:478-486.

Scott, B.D. 1978. Phytoplankton distribution and light attenuation in Port Hacking estuary. Aust. J. Mar. Freshwater Res. 29:31-44.

Seddon, B. 1972. Aquatic macrophytes as limnological indicators. Freshwater Biol. 2:107-130.

Seitzinger, S., S. Nixon, M.E.Q. Pilson, and S. Burke. 1980. Denitrification and N_2O production in near-shore marine sediments. Geochimica et Cosmochimica Acta 44:1853-1860.

Shoaf, W.T. and B.W. Lium. 1976. Improved extraction of chlorophyll a and b from algae using Dimethyl Sulfoxide. Limnol. Oceanogr. 21:926-928.

Sinclair, L.R. and R.B. Forbes. 1980. Nutrient removal from drainage waters with systems containing aquatic macrophytes. American Society of Agricultural Engineers 23:1189-1194.

Smith, E.V. and H.S. Swingle. 1941. The use of fertilizer for controlling several submerged aquatic plants in ponds. Trans. Amer. Fish. Soc. 71:94-101.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14:799-801.

Sørensen, J. 1978. Capacity for denitrification and reduction to nitrate to ammonia in a coastal marine sediment. Appl. Environ. Microbiol. 35:301-305.

Stevenson, J.C. and N.M. Confer. 1978. Summary of available information on Chesapeake Bay submerged vegetation. Fish and Wildlife Service, Office of Biological Services - 78/66.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. Fish. Res. Bd. of Canada Bull. 167 (2nd edition).

Stuckey, R.L. 1978. The decline of lake plants. Natural History 87:66-69.

Valderrama, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry 10:109-122

Westlake, D.F. 1978. Rapid exchange of oxygen between plant and water. Verh. Int. Ver. Theor. Angew. Limnol. 20:2362-2367.

Wheeler, P.A. and W.J. North. 1981. Nitrogen supply, tissue composition and frond growth rates for Macrocystis pyrifera off the coast of southern California. Marine Biology 64:59-69.

Wolverton, B.C, R.M. Barlow, and R.C. McDonald. 1975. Application of vascular aqutic plants for pollution removal, energy and food production in a biological system. NASA Technical Memorandum TM-X-72726.

Table A-1. Concentrations of ammonium (μM) in the eight experimental ponds during the summer of 1981 for the three nutrient treatments and controls.

DOSAGE	DAYS FOLLOWING TREATMENT	DATE	CONTROL		LOW		MEDIUM		HIGH	
			1	5	3	6	4	8	2	7
Pretreatment	0	6 June	3.2	1.1	1.4	1.0	2.9	1.2	2.7	0.6
	6	12 June	0.5	ND*	0.1	ND	0.1	0	2.6	0.3
	10	16 June	1.6	ND	1.0	ND	0.1	0.2	0.1	0.4
First	3	22 June	2.1	0.4	0.3	0.3	0.4	0.2	0.6	ND
	7	26 June	ND	ND	1.7	0.5	ND	ND	0.4	2.1
Second	0	30 June	0.2	0.1	23.0	17.7	35.1	36.6	64.9	48.9
	3	3 July	0.9	1.1	0.2	0.2	0.4	0.4	1.3	0.1
Third	0	8 July	2.0	0.8	52.2	35.3	25.4	31.1	143.5	90.2
Fourth	0	16 July	0.3	1.1	26.4	24.6	43.4	31.1	18.4	62.5
	1	17 July	ND	0.4	2.1	0.6	10.0	11.6	20.7	37.1
	3	19 July	0.1		0.4	0.1	0.2	0.1	0.7	2.3
	7	23 July								
Fifth	0	28 July	2.5	0.7	ND	7.2	10.4	11.1	2.5	18.1
	1	29 July								
	3	31 July	2.7	ND	ND	ND	0.3	0.5	4.1	0.7
Sixth	0	4 August	0.1	ND	65.4	72.3	121.4	33.2	272.2	271.7
	3	7 August	ND	0.6	ND	0.2	5.4	0.2	25.9	31.9

*ND = not detectable (<0.1 μM)

Table A-2. Concentrations of nitrate plus nitrite (μM) in the eight experimental ponds during the summer of 1981 for the three nutrient treatments and controls.

DOSAGE	DAYS FOLLOWING TREATMENT	DATE	CONTROL		LOW		MEDIUM		HIGH	
			1	5	3	6	4	8	2	7
Pretreatment	0	6 June	2.1	1.5	3.0	2.4	2.9	2.2	2.4	2.2
	6	12 June	0.1	0.4	ND	0.7	0.8	ND	1.0	0.4
	10	16 June	0.9	0.3	0.2	0.1	0.2	ND	0.4	0.1
First	3	22 June	2.6	0.5	0.9	3.1	31.2	17.6	59.7	44.8
	7	26 June	ND*	ND	ND	ND	0.6	0.6	24.5	30.1
Second	0	30 June	ND	ND	24.5	17.3	36.9	36.6	77.7	73.1
	3	3 July	ND	ND	0.2	ND	1.7	0.2	33.1	24.2
Third	0	8 July	0.4	0.4	61.3	59.4	37.1	55.0	172.4	124.7
Fourth	0	16 July			21.1	28.1	54.3	36.4	96.7	86.0
	1	17 July	0.2	0.1	5.8	8.1	54.3	29.5	56.3	78.2
	3	19 July	ND	0.3	ND	ND	ND	ND	13.8	48.7
	7	23 July								
Fifth	0	28 July	0.2	1.4	14.3	18.3	27.9	35.9	79.2	89.1
	1	29 July	1.1	1.1	3.9	9.9	5.7	18.3	50.4	68.2
	3	31 July	0.4	1.5	0.9	0.6	9.4	14.3	28.5	28.3
Sixth	0	4 August	1.0	0.2	98.8	52.3	117.8	51.8		
	3	7 August	0.3	2.8	0.5	0.8	10.1	6.3	53.9	79.5

*ND = not detectable (<0.1 μM)

Table A-3. Concentrations of dissolved inorganic phosphorus (µM) in the eight experimental ponds during the summer of 1981 for the three nutrient treatments and controls.

DOSAGE	DAYS FOLLOWING TREATMENT	DATE	CONTROL		LOW		MEDIUM		HIGH	
			1	5	3	6	4	8	2	7
Pretreatment	0	6 June	.02	ND*	0.07	ND	ND	ND	ND	ND
	6	12 June	ND	ND	ND	ND	ND	ND	ND	ND
	10	16 June	ND	ND	ND	ND	ND	ND	ND	ND
First	3	22 June	.01	ND	ND	ND	ND	ND	ND	ND
	7	26 June	ND	ND	ND	ND	ND	ND	ND	ND
Second	0	30 June	ND	ND	3.5	2.4	4.2	4.9	0.8	6.6
	3	3 July	ND	ND	ND	ND	ND	ND	ND	ND
Third	0	8 July	ND	ND	10.2	3.5	6.1	6.7	35.5	15.2
Fourth	0	16 July	ND	ND	4.1	5.0	7.8	5.4	18.4	10.3
	1	17 July	ND	ND	1.2	4.2	2.0	1.5	7.0	7.3
	3	19 July	ND	ND	0.3	ND	0.3	ND	0.93	1.70
	7	23 July								
Fifth	0	28 July	ND	ND	0.53	2.0	2.87	0.95	6.67	7.70
	1	29 July	ND	ND	0.23	0.83	0.90	0.10	7.40	4.27
	3	31 July	0.23	0.10	0.53	0.27	0.60	0.27	0.10	1.40
Sixth	0	4 August	0.03	2.80	9.10	13.2	21.10	8.30	17.8	5.2
	3	7 August								

*ND - not detectable (<0.01 µM)

Table A-4. Concentrations of total nitrogen (μM) in the eight experimental ponds during the summer of 1981 for the three nutrient treatments and controls.

DOSAGE	DAYS FOLLOWING TREATMENT	DATE	CONTROL		LOW		MEDIUM		HIGH	
			1	5	3	6	4	8	2	7
Pretreatment	0	6 June	23.8	12.0	20.8	47.7	16.1	7.6	31.7	15.1
	6	12 June								
	10	16 June	29.5	19.4	26.0	18.4	17.1	23.0	26.6	17.3
First	3	22 June	29.3		34.9				167.5	
	7	26 June	25.3	23.0	25.7	18.6	25.9	30.3	60.7	54.1
Second	0	30 June								
	3	3 July	29.2		29.9		50.9		111.5	
Third	0	8 July	27.7	25.3	127.9	69.3	68.7	116.1	371.8	
Fourth	0	16 July								
	1	17 July	29.2	23.1	43.0	30.2	60.9	84.8	111.9	171.4
	3	19 July	27.6	21.8	32.9	44.9	45.5	43.3	49.8	117.1
	7	23 July	40.9	35.5	38.4		36.0	97.9	71.4	95.7
Fifth	0	28 July								
	1	29 July								
	3	31 July								
Sixth	0	4 August								
	3	7 August	36.6	22.1	47.6	28.9	41.7	98.6	239.3	158.1

Table A-5. Concentrations of total phosphorus (μM) in the eight experimental ponds during the summer of 1981 for the three nutrient treatments and controls.

DOSAGE	DAYS FOLLOWING TREATMENT	DATE	CONTROL		LOW		MEDIUM		HIGH	
			1	5	3	6	4	8	2	7
Pretreatment	0	6 June	0.5	0.5	0.6	1.0	0.6	0.4	0.9	0.6
	6	12 June								
	10	16 June	0.7	0.5	0.8	0.5	0.6	0.5	1.1	0.5
First	3	22 June	1.3				1.6		5.8	
	7	26 June	0.7	0.7	0.8	0.8	0.6	1.1	1.1	0.8
Second	0	30 June								
	3	3 July	1.1	0	1.5	0.6	2.1	1.2	2.8	6.7
Third	0	8 July	1.2	1.1	11.9	5.9	5.2	9.7	38.6	
Fourth	0	16 July								
	1	17 July	1.1	1.1	2.5	2.7	3.4	5.1	7.7	10.2
	3	19 July	1.1	1.2	1.5	2.4	2.6	3.8	2.9	4.0
	7	23 July	1.3	1.2	1.6	3.8	1.8	1.9	2.4	3.6
Fifth	0	28 July								
	1	29 July								
	3	31 July								
Sixth	0	4 August								
	3	7 August	1.9	1.0	2.5	1.5	2.3	4.6	13.2	9.4

Table B-1. Nitrogen content (%/gdw) in tissues of Potamogeton perfoliatus in eight experimental ponds during summer 1981 for 3 nutrient treatments and controls.*

DATE	Control			Low			Medium			High		
	1	5	Mean	3	6	Mean	4	8	Mean	2	7	Mean
ABOVEGROUND												
4 June		1.70	1.70	2.35		2.35	2.54	2.62	2.58	2.13	2.36	2.25
17 June	1.40 (0.20)	-	1.40 (0.20)	1.53 (0.11)	1.49 (0.27)	1.50 (0.25)	2.09 -	1.63 -	1.86 -	2.04 (0.39)	1.93 (0.18)	1.99 (0.29)
13 July	1.24 (0.20	-	1.24 (0.20)	1.75 (0.35)	1.67 (0.16)	1.71 (0.27)	1.92 (0.46)	1.80 (0.12)	1.96 (0.42)	2.52 (0.21)	2.10 (0.28)	2.31 (0.25)
13 August	1.12 (0.19)	2.05 (0)	1.43 (0.16)	2.00 (0.01)	1.77 (0.12)	1.84 (0.10)	2.24 (0.19)	1.96 (0.13)	2.11 (0.17)	3.14 (0.24)	2.99 (0.28)	3.07 (0.26)
BELOWGROUND												
4 June	1.46	-	-	1.56 (0.25)	1.42 -	1.54 (0.23)	1.49 (0.27)	1.49 (0.18)	1.49 (0.25) (0.09)	1.54 (0.18)	1.43 (0.15)	1.49 (0.17) (0.06)
17 June	1.15 (0.61)	-	-	-	0.87 (0.21)	-	-	-	-	1.21 (0.31)	1.27 (0.35)	1.24 (0.31)

*Given are means and (SD) for 3-6 plants in each pond and pooled mean and (SD) for data combined from replicate ponds.

Table B-2. Ratio of carbon:nitrogen in tissues of *Potamogeton perfoliatus* in eight experimental ponds during summer 1981 for 3 nutrient treatments and controls.*

	Control			Low			Medium			High		
	1	5	Mean	3	6	Mean	4	8	Mean	2	7	Mean
ABOVEGROUND												
4 June												
17 June	15.8 (0.3)			20.9 (0.1)	16.9 (2.5)	18.0 (2.3)	16.2 -	21.2 -	18.7 -	13.1 (1.1)	16.1 (1.8)	14.6 (1.5)
13 July	20.5 (2.6)			16.4 (0.9)	15.4 (0.6)	15.9 (0.8)	13.6 (1.2)	13.6 (0.4)	13.6 (1.1)	11.7 (0.4)	10.9 (0.5)	11.4 (0.4)
13 August	19.1 (2.0)	16.7 (3.7)	18.4 (2.5)	15.4 (1.0)	15.9 (2.0)	15.7 (1.7)	13.5 (1.9)	13.5 (1.9)	13.5 (1.9)	10.1 (0.5)	10.2 (2.3)	10.1 (1.6)
BELOWGROUND												
4 June												
17 June												

*Given are means and (SD) for 3-6 plants in each pond and pooled mean and (SD) for data combined from replicate ponds.

Table C-1. Light attenuation coefficients (m^{-1}) in experimental ponds during the summer of 1981 for three nutrient treatments and controls.

DOSAGE	DAYS FOLLOWING TREATMENT	DATE	CONTROL		LOW		MEDIUM		HIGH	
			1	5	3	6	4	8	2	7
Pretreatment	0	6 June	1.14	0.74	0.71	0.97	0.82	0.92	0.86	1.08
	6	12 June	0.93	0.74	0.58	0.77	0.83	1.08	0.97	0.82
First	3	22 June	1.53	1.22	1.24	1.34	1.11	1.62	2.74	2.52
Fourth	3	19 July	0.98	1.72	0.22	1.59	1.10	1.17	1.48	2.30
	7	23 July	1.05	0.94	1.25	1.27	1.23	1.72	1.61	4.16
Fifth	3	31 July	0.97	0.77	0.93	0.95	1.00	1.66	1.63	7.20

Table C-2. Concentrations of chlorophyll-*a* (g/) in the eight experimental ponds during the summer of 1981 for the three nutrient treatments and controls.

DOSATE	DAYS FOLLOWING TREATMENT	DATE	CONTROL 1	CONTROL 5	LOW 3	LOW 6	MEDIUM 4	MEDIUM 8	HIGH 2	HIGH 7
Pretreatment	10	16 June	1.1	1.5	2.4	0.4	1.0	0.3	4.1	1.0
First	3	22 June	0.5	0.7	0.9	1.1	1.2	4.3		5.7
Second	0	30 June	0.7	0.3	0.5	0.2	0.2	0.6	19.0	0.6
	3	3 July[a]	0.8	0.5	3.3	0.5	1.0	0.7	1.0	
Third	0	8 July	0.5	0.2	0.3	0.6	0.3	1.0	5.7	151.8
Fourth	0	16 July	0.5	0.5	2.1	1.8	1.1	1.9	2.5	2.0
	3	19 July	0.9	0.4	5.9	21.0	8.4	9.1	64.9	4.1
	7	23 July	1.7	3.5	28.6	1.2	1.8	2.2	9.2	210.4
Sixth	3	7 August	4.8	0.2	3.9	5.2	0.6	4.8	8.2	9.8

[a] Only one measurement per pond - middle pier.

Table C-3. Concentrations of total suspended solids (mg/) in the eight experimental ponds during the summer of 1981 for the three nutrient treatments and controls.

DOSAGE	DAYS FOLLOWING TREATMENT	DATE	CONTROL		LOW		MEDIUM		HIGH	
			1	5	3	6	4	8	2	7
Pretreatment	0	6 June	19.2	13.6	14.6	15.6	15.2	16.4	17.7	17.1
	6	12 June	19.3	14.4	15.4	17.4	16.4	19.4	18.4	19.6
	10	16 June	17.3	14.4	17.5	16.5	14.3	16.9	16.2	17.0
First	3	22 June	24.6	23.6	18.7	22.7	20.1	24.6	37.0	31.2
Second	0	30 June	17.9	13.6	13.7	17.4	16.4	14.1	18.1	18.0
Third	0	8 July	16.6	11.9	11.6	17.0	14.7	17.2	17.6	22.3
Fourth	3	19 July	22.4	14.8	22.8	42.0	40.9	10.1	21.4	32.4
	7	23 July								
Sixth	3	7 August	36.6	29.3	34.2	36.6	32.2	47.4	46.3	21.9

Table D-1. Summary of biomass estimates (g dry wt/m^2) for submerged aquatic plants in experimental ponds subjected to nutrient additions.*

Date: Species	Value	Nutrient Treatment											
		Control			Low			Medium			High		
		1	5	Mean #	3	6	Mean #	4	8	Mean #	2	7	Mean #
4 June:													
Potamogeton	-	27.0	6.6	17.7	47.2	11.6	29.4	33.2	45	35.2	102.5	49	78.2
	(SD)	(29.9)	(14.8)	(24.3)	(41.3)	(14.4)	(31.0)	(16.5)	(-)	(16.5)	(66.7)	(38.8)	(56.1)
Ruppia	-	56.2	159	107.6	32.2	61.8	47.5	62.2	0	51.8	5.8	11.0	8.2
	(SD)	(78.7)	(73.3)	(24.3)	(74.1)	(71.1)	(72.6)	(118.4)	(-)	(118.4)	(14.3)	(20.9)	(17.54
Total	-	83.7	165.6	120.9	80.3	80.7	80.5	95.4	45.0	87.0	108.3	60.0	86.4
	(SD)	(62.0)	(62.9)	(62.4)	(62.9)	(65.7)	(64.3)	(107.8)	(-)	(107.8)	(65.7)	(30.8)	(53.1)
17 June:													
Potamogeton	-	101.5	0.5	51.0	40.9	49.8	44.9	40	117.2	78.6	187.2	54.0	120.6
	(SD)	(72.5)	(0.8)	(51.3)	(44.0)	(53.8)	(48.6)	(45.6)	(88)	(70.1)	(51.8)	(34.3)	(43.9)
Ruppia	-	84.5	267.3	175.9	63.2	21.2	44.7	53.5	5.0	29.3	1	2.7	1.8
	(SD)	(131.3)	(133.5)	(132.4)	(72.9)	(43.1)	(61.4)	(70.2)	(7.04)	(49.9)	(2.5)	(5.6)	(4.3)
Total	-	186.0	267.8	226.9	102.3	72.6	88.8	93.5	122.2	107.8	188.2	56.7	122.4
	(SD)	(62.0)	(134.3)	(104.6)	(43.0)	(46.8)	(44.7)	(68.9)	(85.1)	(77.4)	(50.7)	(37.3)	(44.5)
13 July:													
Potamogeton	-	133.3	0	66.6	109.2	106.5	107.8	82	32.8	57.4	198	140.2	169.1
	(SD)	(107.2)	(0)	(75.8)	(90.9)	(83.6)	(87.3)	(71.5)	(47.9)	(60.9)	(70.1)	(58.6)	(64.6)
Ruppia	-	85.4	255.7	170.5	50.8	51.2	51	130.3	68	99.2	0	3.34	1.7
	(SD)	(129.1)	(110.4)	(120.1)	(112.3)	(61.5)	(90.5)	(159.6)	(84.2)	(127.6)	(0)	(6.36)	(4.5)
Total	-	218.7	255.7	237.2	160.9	158.5	159.7	212.3	100.8	156.6	200.6	144.0	172.3
	(SD)	(64.7)	(110.4)	(90.5)	(88.3)	(51.6)	(72.4)	(149.3)	(71.0)	(116.9)	(68.9)	(57.0)	(63.2)

Table D-1. Continued.

Date: Species	Value	Nutrient Treatment Control 1	5	Mean #	Low 3	6	Mean #	Medium 4	8	Mean #	High 2	7	Mean #
13 August:													
Potamogeton	-	90.5	3.7	47.1	154	82.2	118.1	129.2	31.5	80.3	106	30.8	78.3
	(SD)	(78.5)	(5.7)	(33.7)	(158.9)	(43.3)	(116.4)	(73.1)	(37.1)	(58)	(33.5)	(36.1)	(46.8)
Ruppia	-	46	262.5	154.3	117.7	38.5	78.1	1.8	10.2	6	0	0	0
	(SD)	(71.3)	(35.6)	(131.3)	(155.7)	(88.6)	(127.6)	(4.0)	(22.8)	(16.1)	(0)	(0)	(0)
Total	-	136.5	266.2	201.3	271.7	120.7	196.2	130.7	40.0	85.3	106.0	50.5	78.3
	(SD)	(45.8)	(36.8)	(41.5)	(117.5)	(49.6)	(90.2)	(76.1)	(34.2)	(59.0)	(55.5)	(36.1)	(46.8)
13 November:													
Potamogeton	-		0		23.1			9.1			0		
	(SD)		(-)		(14.1)			(6.2)			(-)		
Ruppia	-		50.2		0.9			0.3			0		
	(SD)		(19.3)		(1.3)			(0.7)			(-)		
Total	-		50.2		24.0			9.4			0		
	(SD)		(19.3)		(15.1)			(6.8)			(-)		

*Given are mean ($\bar{x}$) and standard deviation (SD) values g dry wt/m^2 from 5 replicate 0.1 m^2 stratified samples.
#Mean and pooled variance of two replicate ponds.

Table D-3. Specific leaf area (cm^2/gdw) of *Potamogeton perfoliatus* and *Ruppia martima* in some of the experimental ponds.

Pond	Date	Section	Potamogeton			Ruppia		
			$\bar{x}$	SD	%C	$\bar{x}$	SD	%C
1	15 June	Tip	124.5	35.4	28	12.4	5.3	43
	1 July	Tip	193.8	52.4	27	71.0	38.3	54
2	16 June	Tip	252.3	54.2	21	49.3	9.1	19
	1 July	Tip	213.6	59.4	28			
3	16 June	Tip	318.9	49.0	15	40.4	6.1	15
	15 July	Tip	273.5	43.1	16			
		Base	210.1	45.1	22			
		Whole	241.8	53.9	16			
7	18 June	Tip	216.0	54.0	25	58.9	14.0	24
	15 July	Tip	251.1	115.1	46			
		Base	192.8	102.6	53			
		Whole	217.0	108.0	50			
8	18 June	Tip	194.2	80.0	41	48.8	13.7	28

Table D-4. Chlorophyll *a* and b concentrations (g/cm^2) and ratios for *Potamogeton perfoliatus* in a low nutrient dosed pond in relation to the dates nutrients were applied to the pond.

Date of Application	Sampling	Chlor *a*	Chlor *b*	a/b
July 8				
	July 14	13.11	2.01	6.53
	July 16	9.74	2.25	4.33
July 17				
	July 23	10.54	2.35	4.48
	July 28	9.35	2.83	3.31
July 29	July 29	10.90	1.53	7.15
	July 31	10.92	2.36	4.62
August 4	August 4	8.36	1.87	4.47
	August 5	7.37	2.39	3.08
	August 6	9.04	2.68	3.38
	August 10	10.99	1.73	6.34

Table D-5. Summary of total epiphytic material (gdw/gdw plant) on submerged vascular plants in experimental ponds subjected to nutrient additions.*

Species Date	Value	Nutrient Treatment Control 1	5	Mean	Low 3	6	Mean	Medium 4	8	Mean	High 2	7	Mean
Potamogeton													
17 June	-	2.57	-	2.57	0.14	2.00	1.07	1.20	1.02	1.11	2.55	2.40	2.49
	(SD)	(0.09)	-	(0.09)	(0.05)	(0.55)	(1.08)	(0.51)	(0.30)	(0.39)	(0.27)	(0.12)	(0.22)
13 July	-	3.93		3.93	1.32	4.50	2.91	4.80	4.97	4.84	3.15	3.95	3.63
	(SD)	(0.78)		(0.78)	(0.38)	(0.62)	(1.80)	(0.98)	(3.48)	(2.28)	(0.60)	(1.30)	(1.04)
13 August	-	4.17		4.17	4.44	4.03	4.23	2.87	3.68	3.28	4.89	3.78	4.33
	(SD)	(1.11)		(1.11)	(0.62)	(1.15)	(0.86)	(1.40)	(1.31)	(1.30)	(0.17)	(0.80)	(0.82)
Ruppia													
17 June	-	3.08	0.09	1.59	0.09	3.24	1.67	0.40	4.67	2.96	-	-	
	(SD)	(0.72)	(0.07)	(1.70)	(0.05)	(0.47)	(1.75)	(0.36)	(1.74)	(2.64)			
13 July	-	3.52	0.09	1.80	0.87	3.43	2.15	4.47	4.74	4.60			
	(SD)	(1.59)	(0.02)	(2.13)	(0.20)	(0.68)	(1.47)	(0.89)	(1.50)	(1.12)			
13 August	-	3.96	0.10	2.03	1.88	3.00	2.44	5.24	5.11	5.17			
	(SD)	(0.51)	(0.04)	(2.14)	(0.65)	(1.38)	(1.14)	(1.49)	(1.27)	(1.22)			

Table E-1. Summary of estimates of apparent production (P_a), dark respiration (R_n), and gross production (P) (mg O_2 $gdw^{-1}h^{-1}$) for _Potamogeton perfoliatus_ and their epiphytes by measuring O_2 production for plant epiphyte groups together and separated.*

Source: Date	Plant Segment	Potamogeton			Epiphyte			Total			P:R Ratio		
		P_a	R_1	P	P_a	R	P	P_a	R	P	SAV	Epi- phytes	Total
LOW NUTRIENT POND:													
16 June	Tip	5.48	1.36	6.84	0	1.07	1.07	5.48	2.43	7.91	4.0	0	2.3
15 July	Tip	9.20	0.30	9.50	2.09	0.90	2.99	11.29	1.20	12.49	30.7	2.3	9.4
	Base	4.80	0.90	5.70	2.06	0.79	2.85	5.70	1.69	7.39	5.3	2.6	3.4
5 August	Tip	9.79	2.45	12.24	2.77	0.77	3.54	12.56	3.22	15.78	4.0	3.6	3.9
	Base	6.10	1.78	7.88	1.08	0.60	1.68	7.18	2.38	9.56	3.4	1.8	3.0
HIGH NUTRIENT POND:													
18 June	Tip	3.90	0.40	4.30	0	1.79	1.79	3.90	2.19	6.09	9.8	0	1.8
15 July	Tip	2.87	1.09	3.96	3.72	0.71	4.43	6.59	1.80	8.39	2.6	5.2	3.7
	Base	0.61	6.03	6.64	5.69	1.11	6.80	6.30	7.13	13.43	0.1	5.1	0.8
5 August	Tip	0	0.25	0.25	8.95	5.57	14.52	8.79	5.82	14.61	0	1.6	1.5
	Base	0	1.65	1.65	5.49	2.61	8.10	5.31	4.26	9.57	0	2.1	1.2

*Experiments performed in light and dark BOD bottles.
#Plant segment was either the 0-15 cm apical portion (tip) or the lower stem, 15-30 cm from apex (base).

Table E-2. Epiphyte biomass and net productivity, and productivity of the host plant *Ruppia maritima* for the upper (0-15 cm) and lower (+15 cm) sections of the plant. Total production is expressed per gdw of the macrophyte.

Plant Section	Epiphyte Biomass (gdw)	*Ruppia* Production (mg C $gdw^{-1}h^{-1}$)	Epiphyte Production (mg C $gdw^{-1}h^{-1}$)		Total Production (mg C $gdw^{-1}h^{-1}$)
			Per Epiphyte	Per SAV	
0-15	-	3.82	-	-	-
	0.37	2.13	0.51	0.19	2.32
	0.48	2.31	0.80	0.38	2.69
	0.63	3.23	0.57	0.36	3.59
15+	-	2.56	-	-	-
	0.32	0.62	0.78	0.25	0.87
	0.40	0.91	0.86	0.34	1.25
	0.45	0.66	0.64	0.29	0.95

Table E-3. Epiphyte biomass and productivity, and productivity of the host plant *Potamogeton perfoliatus* for the upper (0-15 cm) and lower (+15 cm) sections of the plant. Total production is expressed per gdw of the macrophyte.

Plant Section (cm)	Epiphyte Biomass (g/gdw(SAV))	Potamogeton Production (mgC gdw^{-1} h^{-1})	Epiphyte Production Per Epiphyte (mgC gdw^{-1} h^{-1})	Epiphyte Production Per SAV (mgC gdw^{-1} h^{-1})	Total Production (mgC gdw^{-1} h^{-1})
0-15	0.29	5.09	0.15	0.04	5.13
	1.73	5.31	0.97	1.68	6.99
	1.90	3.44	1.59	3.02	6.46
	2.39	4.67	1.03	2.46	7.13
	3.76	4.25	0.94	3.53	7.78
	5.22	5.44	0.57	2.98	8.42
	6.13	1.52	0.92	5.64	7.16
15+	0.27	2.32	0.08	0.02	2.34
	0.77	2.00	1.45	1.12	3.12
	1.25	3.12	0.94	1.18	4.30
	1.39	0.96	1.02	1.42	2.38
	1.68	2.58	1.02	1.71	4.29
	3.84	0.85	0.56	2.15	3.00
	5.44	2.08	0.71	3.86	5.94

Table F-1. Apparent production (P_a) for total communities in experimental ponds based on dawn/dusk readings of dissolved oxygen (gO_2 m^{-3} d^{-1}).

Date	Treatment							
	Control		Low		Mid		High	
	1	5	3	6	4	8	2	7
5 June	7.16	6.34	5.53	4.62	5.19	5.50	-	5.17
16 June	5.89	6.31	6.53	4.53	4.33	6.48	5.94	3.40
23 June	4.69 (0.32)	9.19 (1.88)	6.39 (0.84)	6.38 (0.71)	5.77 (0.36)	6.28 (1.41)	5.35 (0.23)	7.80 (0.70)
24 June	4.53 (0.54)	4.65 (1.22)	5.35 (0.34)	4.92 (0.36)	6.55 (0.41)	5.35 (0.46)	7.05 (0.26)	4.30 (0.15)
16 July	3.09 (0.65)	3.84 (0.18)	3.75 (0.38)	2.06 (0.67)	3.18 (0.27)	2.22 (0.16)	4.33 (0.39)	3.55 (0.95)
17 July	7.83 (2.11)	8.03 (1.21)	8.59 (0.66)	7.52 (1.40)	10.26 (2.09)	11.31	10.06 (0.67)	9.52 (1.85)
23 July	8.89 (0.23)	7.43 (1.71)	6.14 (0.50)	5.70 (0.96)	6.23 (0.40)	6.64 (0.70)	9.18 (1.09)	9.42 (0.39)
30 July	9.47 (0.96)	7.95 (0.53)	8.59 (1.01)	7.87 (1.37)	10.21 (1.50)	11.88 (2.19)	8.57 (0.70)	17.28 (4.02)
31 July	8.35 (1.47)	7.17 (1.25)	7.49 (0.39)	6.45	8.83 (0.16)	8.75 (0.98)	3.17	12.46 (1.96)
10 August	7.36 (0.56)	4.71 (0.32)	2.64 (0.33)	5.13 (1.26)	9.29 (0.51)	9.77 (0.62)	1.09 (0.44)	1.92 (0.17)
11 August	6.52	6.92	2.66	5.24	6.80	5.63	1.46	3.10

Table F2. Nighttime respiration (R_n) for total communities in experimental ponds based on dawn/dusk readings of dissolved oxygen ($gO_2\ m^{-3}\ d^{-1}$).

	Treatment							
	Control		Low		Mid		High	
Date	1	5	3	6	4	8	2	7
15 June	3.24 (0.77)	3.05 (0.47)	3.74 (0.67)	3.45 (0.44)	3.80 (0.36)	4.36 (0.11)	4.26 (1.14)	3.24
23 June	2.75 (0.36)	3.31 (1.58)	3.20 (0.46)	3.13 (0.26)	3.35 (0.13)	3.66 (0.80)	5.64 (0.33)	2.71 (0.61)
16 July	4.85 (0.43)	4.07 (0.33)	5.01 (0.07)	4.62 (0.92)	5.05 (0.31)	5.66 (0.30)	6.93 (0.15)	6.15 (0.54)
23 July	6.55 (0.22)	5.17 (0.75)	4.86 (0.63)	6.04 (0.31)	5.94 (0.25)	6.26 (0.18)	8.60 (0.69)	7.25 (0.24)
30 July	5.37 ( )	4.33 (0.53)	4.55 (0.38)	3.35 (0.27)	4.32 (0.78)	6.09 (1.47)	7.10 (0.13)	6.87 (2.00)
10 August	2.48 (0.57)	-	0.63 (0.22)	3.65 (0.69)	3.78 (0.89)	3.76 (0.18)	3.05 (0.20)	3.06 (0.11)

Table F-3. Ratios of apparent production:nighttime respiration (Pa:Rn) for total communities in experimental ponds based on dawn/dusk readings of dissolved oxygen.

Date	Treatment							
	Control		Low		Mid		High	
	1	5	3	6	4	8	2	7
23 June	1.71	2.78	2.00	2.04	1.72	1.72	0.95	2.88
24 June	1.65	1.40	1.67	1.57	1.96	1.46	1.25	1.59
16 July	0.64	0.94	0.75	0.45	0.63	0.39	0.62	0.58
17 July	1.61	1.97	1.71	1.63	2.03	2.00	1.45	1.55
23 July	1.36	1.44	1.26	0.94	1.05	1.06	1.07	1.30
30 July	1.76	1.84	1.89	2.35	2.36	1.95	1.21	2.52
31 July	1.55	1.66	1.65	1.93	2.04	1.44	0.45	1.81
10 August	2.97	-	4.19	1.41	2.46	2.60	0.36	0.63
11 August	2.63	-	4.22	1.44	1.80	1.50	0.48	1.01

CHAPTER IX

THE DECLINE OF SUBMERGED VASCULAR PLANTS IN UPPER CHESAPEAKE BAY: SUMMARY OF RESULTS CONCERNING POSSIBLE CAUSES[1]

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ABSTRACT

This paper provides a summary and synthesis of research conducted to investigate possible causes of the decline in abundance of submerged aquatic vegetation (SAV) in upper Chesapeake Bay beginning in the late 1960's. Three factors potentially were emphasized in this study: runoff of agricultural herbicides; erosional inputs of fine-grain sediments; nutrient enrichment and associated algal growth. Widespread use of herbicides in the estuarine watershed occurred contemporaneous with the SAV loss; however, extensive sampling of estuarine water and sediments during 1980-81 revealed that typical bay concentrations of herbicides (primarily atrazine) rarely exceeded 2 ppb. On two occasions relatively high values (20-45 ppb) were observed for brief (2-4 h) periods in a small cove following runoff events. Short (2-6 h) and long (4-6 wk) term experiments indicated that ephemeral phytotoxic effects would be expected in response to these highest herbicide concentrations followed by rapid recovery. However, normal concentrations ($<$ 5 ppb) had little measurable effect on plants. Historical increases in turbidity have been documented for some bay tributaries since the 1940's. During our study light (PAR) attenuation by suspended fine-grain sediments contributed more to total turbidity in bay shallows ($<$ 1.5 m) than did phytoplankton chlorophyll a. Diel cycles of PAR available in SAV beds indicated that plant photosynthesis was light-limited for much of the day, and PAR often fell below the compensation level (I_C) needed for minimal plant growth. Although some SAV species exhibited considerable ability to adapt to reduced light by such mechanisms as increased pigmentation and stem elongation, increased turbidity has probably reduced overall depth distributions of SAV markedly. Effects of the continual increase in nutrient enrichment of the bay (documented since 1930) were tested by experimentally fertilizing pond mesocosms at levels common to the upper estuary. Moderate to high nutrient loadings resulted in significant increases in growth of epiphytic and planktonic algae and decreases in SAV production, as well as premature seasonal senescence of fertilized plant populations. Direct measurements demonstrated the inhibitory effect of epiphytic growth on SAV photosynthesis, due largely to light attenuation. The results of these various experiments were synthesized into an ecosystem simulation model which demonstrated the relative potential contributions of the 3 factors to SAV declines, where nutrients > sediments > herbicides. Other factors and mechanisms are also discussed along with possible resource managements options.

INTRODUCTION

It is widely recognized that submerged vascular plants play an important role in the ecology of littoral regions of lakes, estuaries and oceans (1, 2, 3). While a number of studies have noted the ability of these plant communities to attenuate variability of nutrient, sediment and production cycles (4, 5, 6), several such communities have themselves undergone extreme fluctuations in distribution and abundance. For example, in the mid 1930's a widespread die-off of the seagrass, *Zostera marina*, was well documented throughout the North Atlantic coastal regions (7). The cause of this occurrence has never been unequivocally established, although recent suggestions have pointed to subtle climatic shifts (8). Other reports of regional declines in abundance of submerged aquatic vegetation (SAV) have indicated the possible influence of human activities (9, 10).

Few of the reported SAV declines have occurred in estuarine environments and most have involved 1-2 plant species. However, in one of the world's largest estuaries, Chesapeake Bay, a major loss of SAV has continued since the mid 1960's to the present (11, 12, 13). More than 10 species have experienced significant decreases in abundancce, including *Potamogeton perfoliatus*, *P. pectinatus*, *Valisneria americana*, *Zannichellia palustris*, *Ruppia maritima* as well as the marine species *Z. marina* (12). In the upper estuary this decline in native species was preceeded by an invasion of the exotic, *Myriophyllum spicatum* (Fig. 1a) which eventually also died-back (11). Studies of seed and pollen distribution in sediment cores from the upper bay have demonstrated that this dimunition in plant abundance is unprecedented (Fig. 1a) for at least the last century (13). In general, it appears that the recent decline occurred first and with greatest intensity in the brackish waters of the estuary, with *Z. marina* communities in the lower bay being affected less and somewhat later (13).

Numerous mechanisms have been cited as possible causes of this occurrence (12). The concept that natural entrained population cycles or global climatic events might be responsible seems unlikely in view of the range of biological and physiological characteristics for the numerous species involved. In addition, there is no parallel trend in plant abundance apparent in nearby coastal regions (13). Other factors including animal foraging and grazing and major storm events are probably of occasional and local importance, but these are part of the normal milieu to which SAV are exposed and hence are insufficient to explain this abnormal decline. The absence of correlations between distribution of SAV and industrial pollutants renders such anthropogenic wastes an unlikely cause; however, more general changes in water quality associated with diffuse sources (e.g. runoff) do represent a potential explanation(12).

In an extensive review concerning SAV in Chesapeake Bay and elsewhere (12), it was suggested that three categories of environmental changes deserved

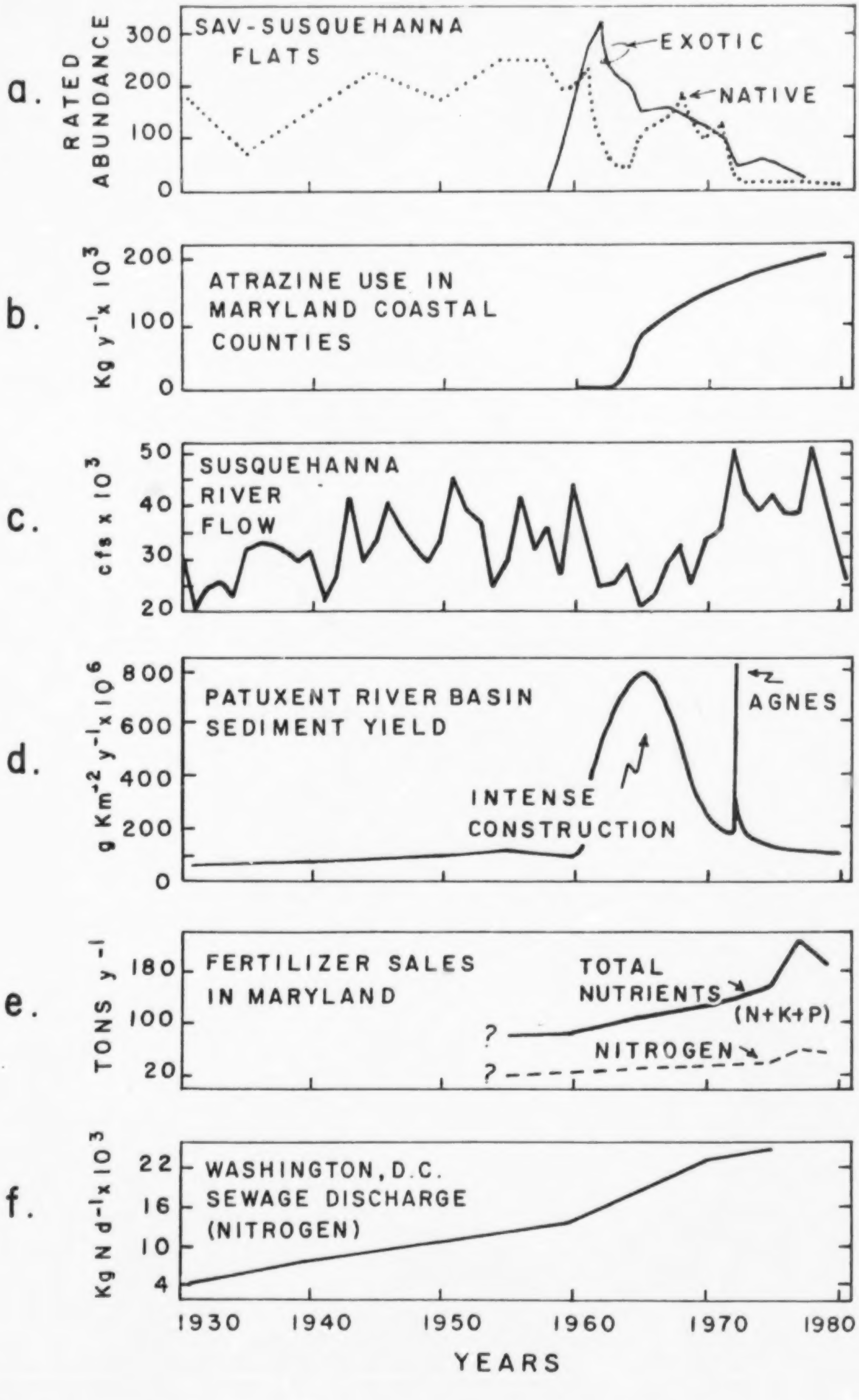
a.
RATED ABUNDANCE
0
100
200
300
SAV-SUSQUEHANNA FLATS
EXOTIC
NATIVE
b.
Kg y^{-1}x 10^3
0
100
200
ATRAZINE USE IN MARYLAND COASTAL COUNTIES
c.
cfs x 10^3
20
30
40
50
SUSQUEHANNA RIVER FLOW
d.
g Km^{-2} y^{-1}x 10^6
0
200
400
600
800
PATUXENT RIVER BASIN SEDIMENT YIELD
INTENSE CONSTRUCTION
AGNES
e.
TONS y^{-1}
20
100
180
FERTILIZER SALES IN MARYLAND
TOTAL NUTRIENTS (N+K+P)
NITROGEN
?
?
f.
Kg N d^{-1}x 10^3
4
10
16
22
WASHINGTON, D.C. SEWAGE DISCHARGE (NITROGEN)
1930
1940
1950
1960
1970
1980
YEARS

Fig. 1. Summary of long-term trends (1930-1980) in selected variables for Chesapeake Bay and its tributaries: a) submerged aquatic vegetation abundance in the upper bay (1958-1976 (11), prior to 1958 (14)); b) use of atrazine in coastal plain counties draining into the bay (12); c) Susquehanna River flow (69); d) idealized sediment yield for Patuxent River basin (28, 29); 3) fertilizer sales in Maryland (35); f) nitrogen in sewage discharge from Washington, D.C. into Potomac River estuary (35, 69).

further attention: increased fine-grain sediments from land erosion; increased algal growth from nutrient enrichment of estuarine waters; and aqueous concentrations of herbicides arising from agricultural runoff.

This paper summarizes the results from recent studies examining possible causes of the SAV decline in upper Chesapeake Bay. Specifically, we report the salient findings of investigations concerning: estuarine distributions, degradation, sorption, and SAV phytotoxicity of selected herbicides; light availability and vertical attenuance by suspended material, distribution of this suspended matter, and photosynthetic responses of SAV to different light regimes; effects of nutrient enrichment on algal growth, and responses of SAV growth and production to elevated levels of planktonic and epiphytic algal biomass. We also synthesize these results toward reconstructing a plausible scenario vis a vis this loss of submerged vascular plants, and we provide comments on resource management options and the future of SAV in upper Chesapeake Bay.

APPROACH TO PROBLEM

In 1978 we initiated a 3-year study to investigate various aspects of the ecology of SAV communities in Chesapeake Bay. While intensive research was conducted at several locations along the estuarine salinity gradient, our work focussed on communities located in the low salinity (5-15°/°°) region. The research was designed to address three general questions: (1) factors potentially responsible for the observed decline in SAV distribution and abundance; (2) SAV community interactions and characteristics; and (3) resource management options.

This research program was organized in a hierarchical fashion to deal with the complexity of the ecosystems studied in addressing these questions. Depicted in Fig. 2 is the manner in which (c) specific research approaches were integrated into (b) broad research objectives which were, in turn, related to (a) research questions (15, 16). General objectives of this reserach program involved: the development of mechanistic relationships among ecological factors through controlled experimentation; the interpretation of these results in terms of actual conditions in nature; and the establishment of a framework to extend specific results in to a general context. The specific research approaches employed ranged from shortterm laboratory experiments to seasonal mesurements in the estuary and experimental ponds, with ecological modeling at all levels for analysis, interpretation and generalization. In the following sections we review the results of our field, laboratory and modeling studies to evaluate the possible roles of herbicides, suspendable sediments and nutrients in the SAV decline in the bay.

HERBICIDE INPUTS, FATE AND PHYTOTOXICITY

For the last two decades the most widely used herbicide in the Chesapeake Bay watershed (and particularly in the surrounding coastal plain) has

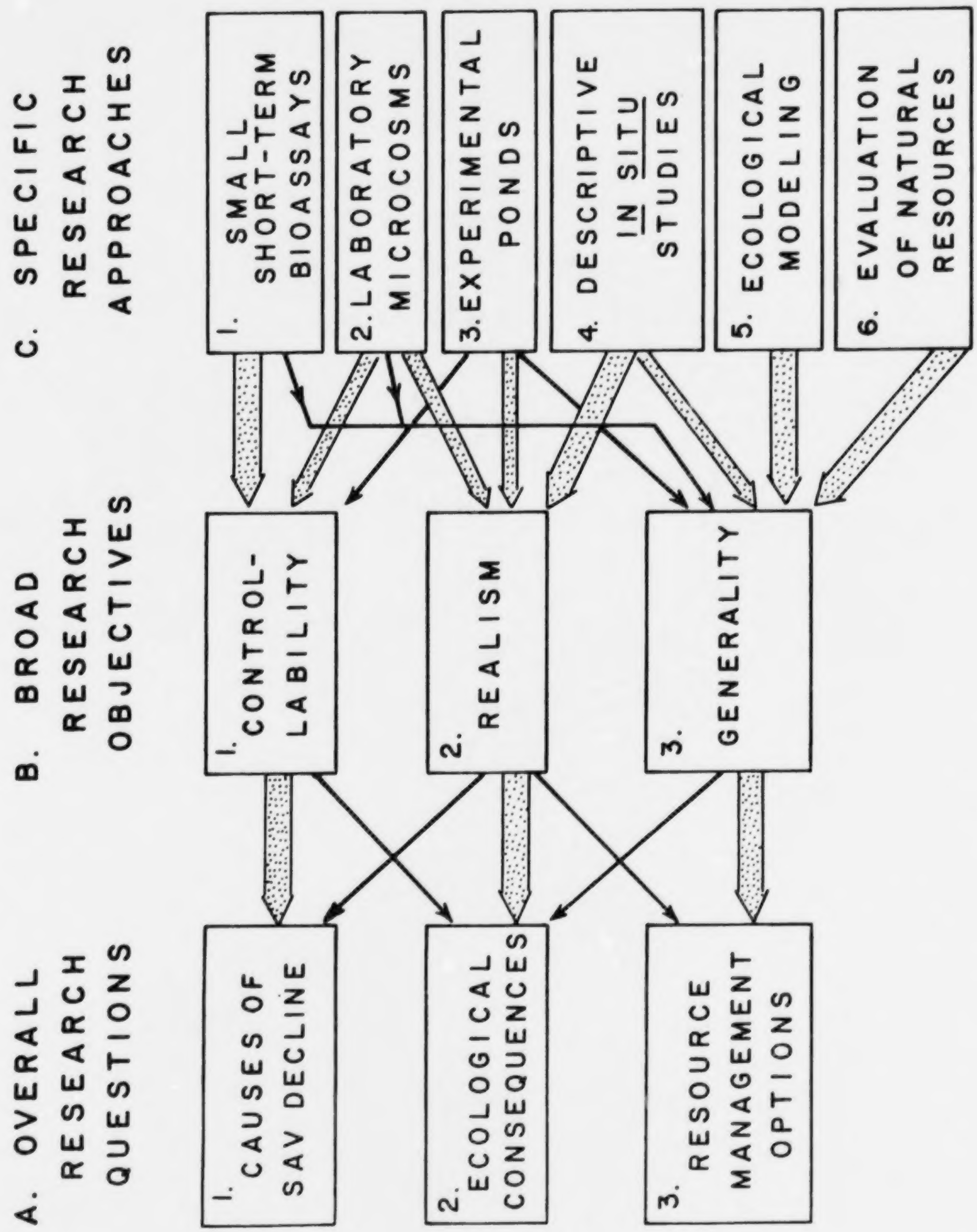
A. OVERALL RESEARCH QUESTIONS
1. CAUSES OF SAV DECLINE
2. ECOLOGICAL CONSEQUENCES
3. RESOURCE MANAGEMENT OPTIONS
B. BROAD RESEARCH OBJECTIVES
1. CONTROL-LABILITY
2. REALISM
3. GENERALITY
C. SPECIFIC RESEARCH APPROACHES
1. SMALL SHORT-TERM BIOASSAYS
2. LABORATORY MICROCOSMS
3. EXPERIMENTAL PONDS
4. DESCRIPTIVE IN SITU STUDIES
5. ECOLOGICAL MODELING
6. EVALUATION OF NATURAL RESOURCES

Fig. 2. Schematic flow chart illustrating the relationships between specific research approaches, broad research objectives and overall research questions. Three thicknesses of arrows suggest levels of influence for these items.

been atrazine (12, 17). Since its introduction to the region in the early 1960's, its use, which is particularly important for corn crops, has grown steadily to the present (Fig. 1b). Atrazine was thus selected as the primary compound for intensive investigation. Our research also considered the distribution and phytotoxicity of a second important compound, linuron, which is used for weed control in soybean crops.

Herbicide Distribution and Degradation

In general about 0.2-2.0% of atrazine applied to agricultural fields is transported into adjacent waters in runoff, and resulting concentrations in the estuary depend on dilution, dispersion, adsorption and degradation (17, 21, 27). In the open waters of Chesapeake Bay observed concentrations of atrazine and linuron rarely have rarely exceeded 1 ppb (18, 19, 20). In major tributaries such as the Choptank and Rappahanock Rivers concentrations of 5 ppb may occur following a major spring runoff event. Such events can generate transient (2-6 h) concentrations up to about 40 ppb in secondary tributaries and small coves (20, 21, 22).

Our data indicate that atrazine and linuron degrade rapidly in estuarine conditions with half-lives of 1-6 wk (17, 23). In addition, these herbicides exhibit strong tendency for sorption to particles and colloids (20, 24), and degradation of particle-bound atrazine appears to be even more rapid (25). As a result of dilution and degradation, concentrations of atrazine on suspended and deposited sediments in the estuary were seldom greater than 5 μg/kg suggesting little accumulation potential, (20), and moreover, once bound to particles atrazine becomes virtually unavailable for plant uptake (25).

Herbicide Uptake and Phytotoxicity

Using ^{14}C-ring labelled atrazine Jones et al. (in 25) found that uptake by *P. perfoliatus* was rapid, reaching equilibrium within 1 h. The observations that atrazine concentrations were low in sediments (20), combined with the relatively slow translocation of the herbicide measured within plant vascular tissues (25) suggest the primary mode of uptake is foliar. A strong correlation was observed between atrazine uptake and short-term depression of photosynthesis. After a sequence of washes in atrazine-free water, a portion of the incorporated atrazine was released and photosynthesis returned to control levels within 4-8 h (25).

In addition to short-term (2 h) experiments relating photosynthetic depression to atrazine exposure, microcosms containing estuarine water and sediments with *P. perfoliatus* or *M. spicatum* were used to test longer duration (4-6 wk) effects of atrazine and linuron on plant growth and production. At concentrations less than 100 ppb of either herbicide, both plants exhibited a significant trend of photosynthetic recovery within 1-2 wk after initial exposure despite the continued presence of herbicides in microcosm waters (25). Using a linear dose-response model for apparent photosynthesis (or net biomass accumulation) versus the logarithm of initial herbicide concentration, significant ($r^2 \geq 0.93$) relationships were observed for all combinations of herbicides and plants (Fig. 3). Values of $I_{1.0}$ and I_{50} (concentrations resulting in 1% and 50% inhibition of photosynthesis) ranged from about 2-4 ppb and 70-120 ppb, respectively, for the 4 combinations of herbicides and plant species tested (25).

At 5-10 ppb (the upper limit of herbicide concentrations typically occurring in most of the estuarine shallows) a 10-20% loss of photosynthesis would be expected. However, the ephemeral nature of such elevated herbicide levels and the tendency toward rapid photosynthetic recovery in these plants would render such effects short in duration. Overall, atrazine phytotoxicity has been tested for 7 species in Chesapeake Bay in various experiments, and some differences in sensitivity were apparent (25, 26, 27). For example, the exotic species, M. spicatum, exhibited greater tolerance than most native plants, and the seagrass, Z. marina, appeared also to be less sensitive, while the freshwater member of the Hydrocharitaceae, V. americana, was among the least tolerant species tested.

Some concern about the possible phytotoxicity of atrazine metabolites formed during degradation was allayed in recent experiments, again using ^{14}C-labelled compounds (provided by Cipa-Geigy Corporation, Greensboro, NC), where I_{50} values were found to be at least 10 times greater than for the parent compound (25). Thus, while conditions certainly occur sporadically in the estuary where herbicide stress could induce some loss in SAV production, the possibility that herbicides led to the SAV decline seems remote.

SUSPENDABLE SEDIMENTS AND SAV LIGHT RESPONSES

Substantial changes in both sediment yield to and turbidity in Chesapeake Bay and some tributaries have occurred over the last several decades (Fig. 1d). In one major tributary (Patuxent River), there have been periods of increasing and decreasing sediment yields, punctuated by occasional major input events during the last 50 years (14, 28, 29, 30). Overall, it seems that turbidity in this tributary is higher now than 30 years ago (33) even though inputs of fine-grain sediments have recently decreased (29), indicating there is not a simple relationship between these factors.

Light Attenuation due to Suspended Sediments

Laboratory and field experiments were conducted to assess the relative contributions of suspended sediments and chlorophyll-a to diffuse, down-welling light attenuation. Diffuse attenuation of phytosynthetically active radiation, PAR (400-700 nm), due to different concentrations of suspended materials was measured in stirred, opaque-sided, cylindrical chambers (60 cm dia, 120 cm ht) by observing PAR at 10 cm vertical intervals. Known amountd of dried, sieved (62 μ) sediment or algal culture suspensions were added to the chambers to develop relations between attenuation coefficient (k) and suspended concentrations. The slope of k versus algal biomass (in terms of dry wt.) was found to be about twice that for k versus inorganic suspended sediments due to the high absorbance of algal pigments at PAR wavelengths (31). However, the relative ranges of these two suspensoids typical of shallow estuarine environments

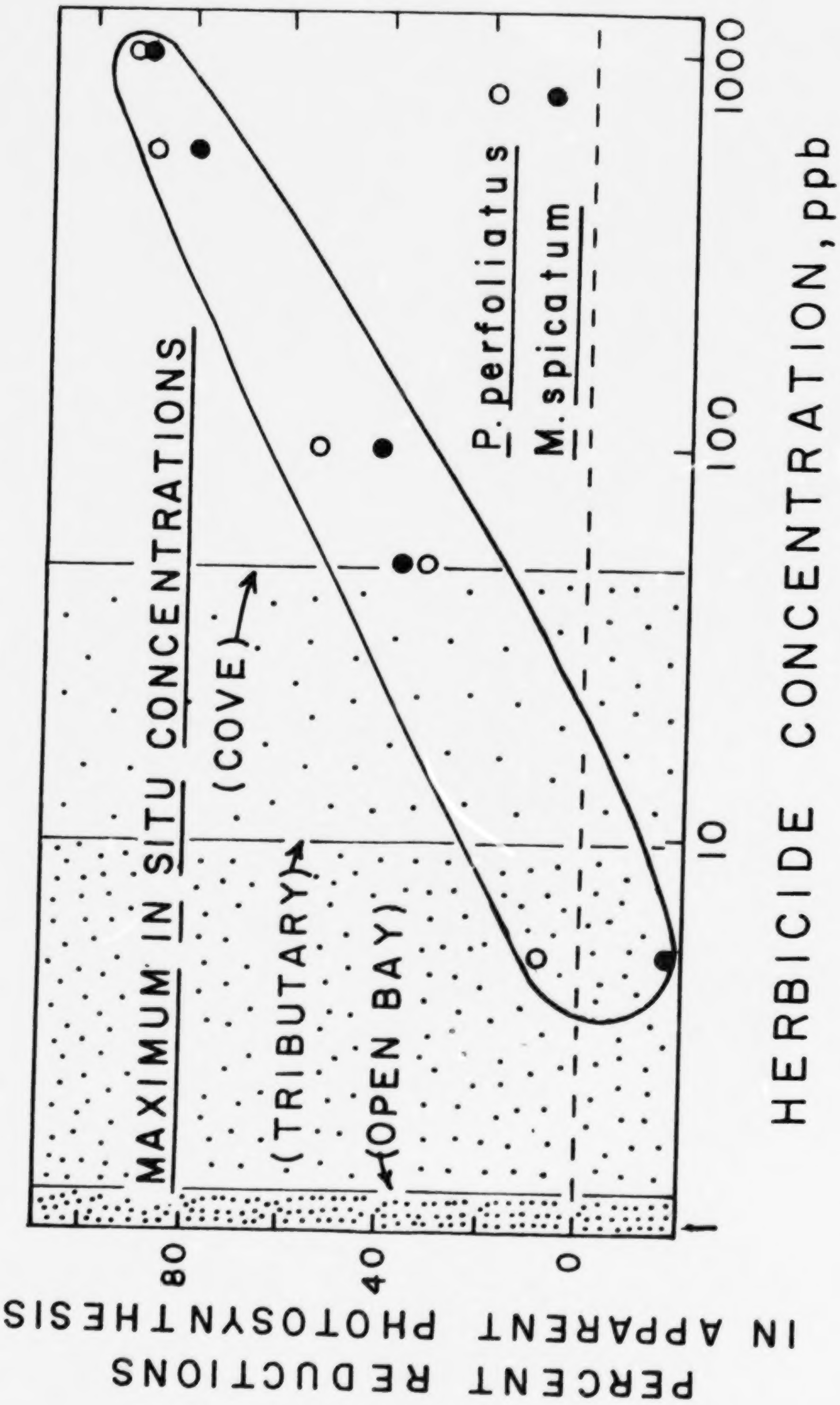
PERCENT REDUCTIONS IN APPARENT PHOTOSYNTHESIS
80
40
0
HERBICIDE CONCENTRATION, ppb
1
10
100
1000
MAXIMUM IN SITU CONCENTRATIONS
(COVE)
(TRIBUTARY)
(OPEN BAY)
○ P. perfoliatus
● M. spicatum

Fig. 3. Reduction in apparent photosynthesis (%) for two species of submerged aquatic vegetation resulting from treatment with the herbicide atrazine at 5 initial concentrations. Three degrees of shading represent (from heavy to light) maximum *in situ* atrazine concentrations observed in: open Chesapeake Bay waters; main bay tributaries; and a small cove within one tributary (17, 25).

suggest that suspended solids are a far greater component to total turbidity. Concentrations of suspended sediments (seston) often increase from 20-100 mg/ℓ within 1-2 h due to moderate (5-10 m/s) summer winds (25, 31), while chlorophyll-a concentrations typically range from 5-15 μg/ℓ over a tidal cycle (Fig. 4) and are not markedly influenced by wind events. These common excurisons in seston and chlorophyll-a would result in about 50% and 8% reductions in PAR at 50 cm depth, respectively.

To assess in-situ light conditions, measurements of seston, chlorophyll-a, attenuation coefficient, water depth and PAR were made over tidal cycles in littoral areas with and without SAV communities (25, 31). Data from a typical cycle (Fig. 4) indicate that both chlorophyll-a and suspended particulate concentrations were lower within SAV communities than in non-vegetated littoral areas, and the differences were greatest at low slack tide, probably due to settling of particulates in the quiescent waters of the bed. It is evident here that PAR attenuation followed seston consistently at both sites, while not so for chlorophyll-a. Consistent with laboratory data, most (> 95%) light attenuation could be attributed to suspended solids other than chlorophyll-a. Sediments may also be important in light attenuation when they settle from the water column onto SAV leaves. For example, Staver et al. (31) found 80-90% of total epiphytic material (attached to leaves) to be inorganic sediments as opposed to algae or other organic materials in a Chesapeake Bay SAV community. However, there is a continuous cycle of deposition and resuspension of these loosely attached sediments, and the integrated, long-term effect of these processes on photosynthesis is not known.

Light Response of SAV

In laboratory chambers the responses of P. perfoliatus and M. spicatum photosynthesis to light intensity followed a basic rectangular hyperbolic form. Values of I_K (intersection of initial slope and P_{max}) ranged from 200-300 $\mu Em^{-2}s^{-1}$ and were similar to values reported for other SAV species (31). A second important parameter of the photosynthesis-irradiance (P-I) relationship is light compensation point (I_c), which is the PAR intensity at which net production (in excess of respiration) approaches zero. Values of I_c at about 50 $\mu Em^{-2}s^{-1}$ were reported for apical 10 cm growing tips of P. perfoliatus (25). However, I_c values for whole plants (including non-photosynthetic root-rhizome tissue) would be substantially higher, probably in the range of 75-150 $\mu Em^{-2}s^{-1}$. Given the ambient turbidity levels generally observed in the bay ($k > 2/m$) in addition to light reduction associated with epiphytic materials, it would appear that SAV are generally exposed to light regimes approaching I_c. Unless these plants can adjust to such light stress, net growth would not be possible.

In fact, Goldsborough (32) found that at least one species (P. perfoliatus) has considerable ability to adapt to reduced light conditions via morphological changes (such as stem elongation and increased leaf area:weight ratio) and biochemical changes (such as increased chlorophyll-a per leaf area) which influence P-I relationships. However, under extreme shading (< 75 $\mu Em^{-2}s^{-1}$) the capacity of adaptive mechanisms was exceeded, resulting in reductions in new shoots, number of flowering plants, and root storage nodules (which appear

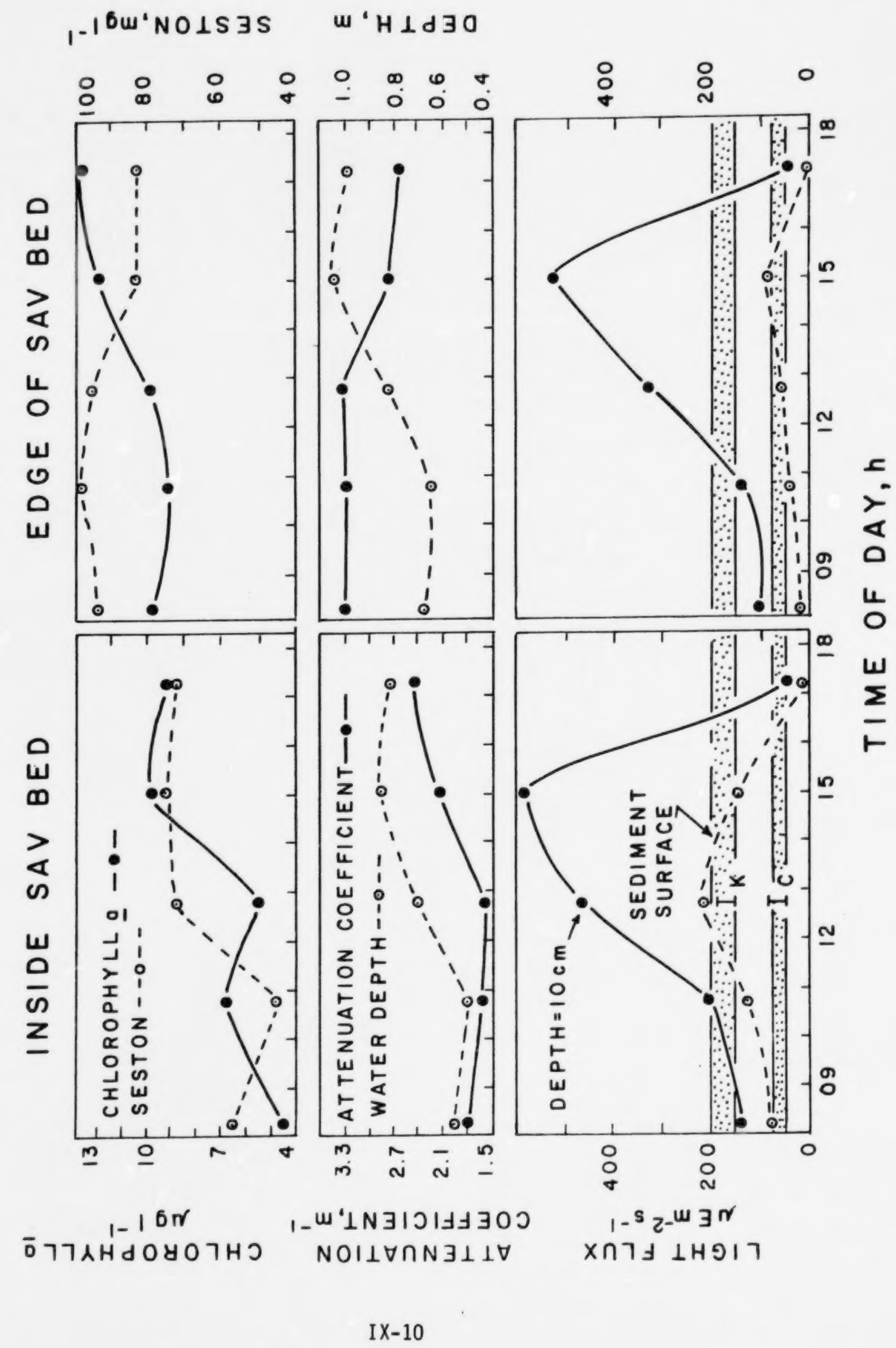
INSIDE SAV BED
EDGE OF SAV BED
CHLOROPHYLL a ●
SESTON ○
ATTENUATION COEFFICIENT ●
WATER DEPTH ○
DEPTH=10cm
SEDIMENT SURFACE
I_K
I_C
CHLOROPHYLL a µg l⁻¹
ATTENUATION COEFFICIENT, m⁻¹
LIGHT FLUX µE m⁻² s⁻¹
SESTON, mg l⁻¹
DEPTH, m
TIME OF DAY, h
13
10
7
4
3.3
2.7
2.1
1.5
400
200
0
100
80
60
40
1.0
0.8
0.6
0.4
09
12
15
18

Fig. 4. Time-course observations for planktonic chlorophyll *a*, total seston, water depth, light attenuation coefficient and light available at 10 cm depth and at the bottom of a 1.0 m (mean depth) water column for a bed of submerged plants (*P. perfoliatus*) and for an adjacent unvegetated area on 22 September 1980. Shaded regions represent typical ranges for I_k (intersection of initial slope and maximum photosynthesis in a photosynthesis-irradiance function) and I_c (compensation light level for 10 cm apical growing tips of plants) (31, 32).

to be overwintering organs, 1).

Overall Effects of Suspendable Sediments on SAV

In the Patuxent Estuary, which formerly contained extensive SAV communities, chlorophyll-a concentrations increased from about 10 to 40μg/ℓ and secchi disc depths decreased from about 0.8 m to 0.35 m (or $k \doteq 1.8 - 4.0$/m, assuming $k = 1.4$/secchi) for spring-summer during the 1960's decade (33). Partitioning these attenuation coefficient values between chlorophyll-a and other particulate and dissolved materials (34) shows about 7% of total attenuation could be attributed to the algal pigment in the early 1960's, while about 14% could be so partitioned for the 1970 data. While the relative importance of phytoplankton attenuation increased over this period, it appears that most light attenuation was still associated with inorganic particulates and other non-chlorophyllous organics.

In any case, the observed increase in turbidity would cause a significant reduction in the depth distribution of SAV. For example, if we take summertime irradiance at the water surface to be 1200 $\mu Em^{-2}s^{-1}$ (a typical value at noon on a sunny Aug-Sep day) and I_c to be 100 $\mu Em^{-2}s^{-1}$, the depth to which sufficient light penetrates to support SAV growth would be reduced from 1.4 m to 0.6 m for the aforementioned scenario in the Patuxent between 1960 and 1970. Similar turbidities were observed (25) throughout littoral zones in upper Chesapeake Bay, suggesting that the depth distribution of SAV has been restricted due to reduced PAR availability.

NUTRIENT ENRICHMENT AND ALGAL-SAV RELATIONS

Watershed inputs of nitrogen (N) and phosphorus (P) to Chesapeake Bay have increased several-fold in the last two decades (Fig. 1 e,f), and aqueous concentrations of inorganic nutrients have likewise increased appreciably in many areas of the estuary (33, 35). Correspondly, phytoplankton populations (as measured by chlorophyll-a) have also expanded during this period (33, 35). Since it appears that submerged vascular plants can obtain their N and P requirements from sediment pore waters (36, 37) which are rich in nutrients, it is expected that nutrient additions to overlying waters would increase SAV growth to a limited extent only. In fact, there are a number of mechanisms where fertilization may lead to decreases in SAV production and abundance. Nutrient enrichment tends to promote algal growth, either phytoplanktonic (38) or epiphytic (10) which in turn can inhibit SAV photosynthesis via reductions in light and molecular transport across SAV epidermis (39, 40). In addition, it has been postulated that increased phytoplankton populations can stimulate growth of animal fouling communities on SAV leaves and other firm, stable substrates (25, 41) leading to similar photosynthetic stress for the plants. In this section we consider this question terms of correlations from field observations and results of nutrient enrichment studies in experimental ponds.

Algal Responses to Nutrient Enrichment

The effects of nutrient enrichment on algal and SAV growth were investigated in 8 experimental ponds (area = 350 m^2; depth = 1.2 m) treated in duplicate with 3 nutrient levels (plus controls) at 8-10 day intervals for a 10 week period during the summer of 1981. Initial nutrient concentrations after dosing were 120, 60 and 30 µM inorganic nitrogen (50% NH_4^+; 50% NO_3^-), with N:P (atomic) ratios of 10:1. Nutrient loading rates at medium dosage were equivalent to runoff from a typical watershed (26% agriculture, 11% residential) draining into a small Chesapeake Bay tributary (42).

Phytoplankton biomass (as chlorophyll-a) generally increased with treatment levels (significantly greater at low and high doses), particularly at the highest nutrient amendments (Fig. 5). A sequence of phytoplankton bloom events were evident in high dose ponds, where chlorophyll-a increased by 1-2 orders of magnitude on several occasions within a 1-2 d period. This response was similar to those previously reported for nutrient enrichment studies (43, 44). Epiphytic algal biomass was significantly higher than controls in all treated ponds (Fig. 5). Levels of epiphytic material in nutrient treated ponds were similar to those observed in the estuary on scenescent plants while control levels were comparable to those occurring early in the SAV growing season (32, 45). Again, this pattern of increased epiphytic algal growth is consistent with results from nutrient enrichment experiments elsewhere (10, 46, 47).

Effects of Algal Growth on SAV

Correlations between PAR attenuation in the water and phytoplankton chlorophyll-a were obtained from measurements in the experimental ponds using only those data where the estimated weight of algal cells comprised a major faction (> 10%) of total seston weight (25). The slope of this relationship (0.0146 m^2mg^{-1}; $r^2 = 0.95$) is quite consistent with observations from deep coastal waters (48) and slightly greater than those derived from laboratory cultures (31, 49). We also obtained a strong relationship between PAR attenuance through a plane (estimated by scraping epiphytes from leaves of known area into a petri dish and measuring PAR attenuation, 50) and epiphytic biomass ($r^2 = 0.87$). The slope of this relation was 0.36% $(mg/cm^2)^{-1}$, which again is comparable to previous reports (10, 50, 51). Direct measurements of SAV photosynthesis (using both ^{14}C uptake and oxygen evolution methods) over a range of epiphytic colonization levels (0-5 gdw epiphyte/gdw SAV) yielded significant correlations with about 50% reduction in photosynthesis at 4 gdw/gdw (25). After 8 wk of nutrient treatment, SAV biomass in medium and high fertilization systems had decreased significantly compared to controls (Fig. 5). While the contribution of the water column to total attenuation (from water surface to plant leaves) was generally small compared to that associated with epiphytic material, without such light reduction due to turbidity, epiphytic growth would have been insufficient to reduce light below compensation levels (I_c). Similar observations on the relative roles of phytoplankton and epiflora have been reported for Danish lakes (51).

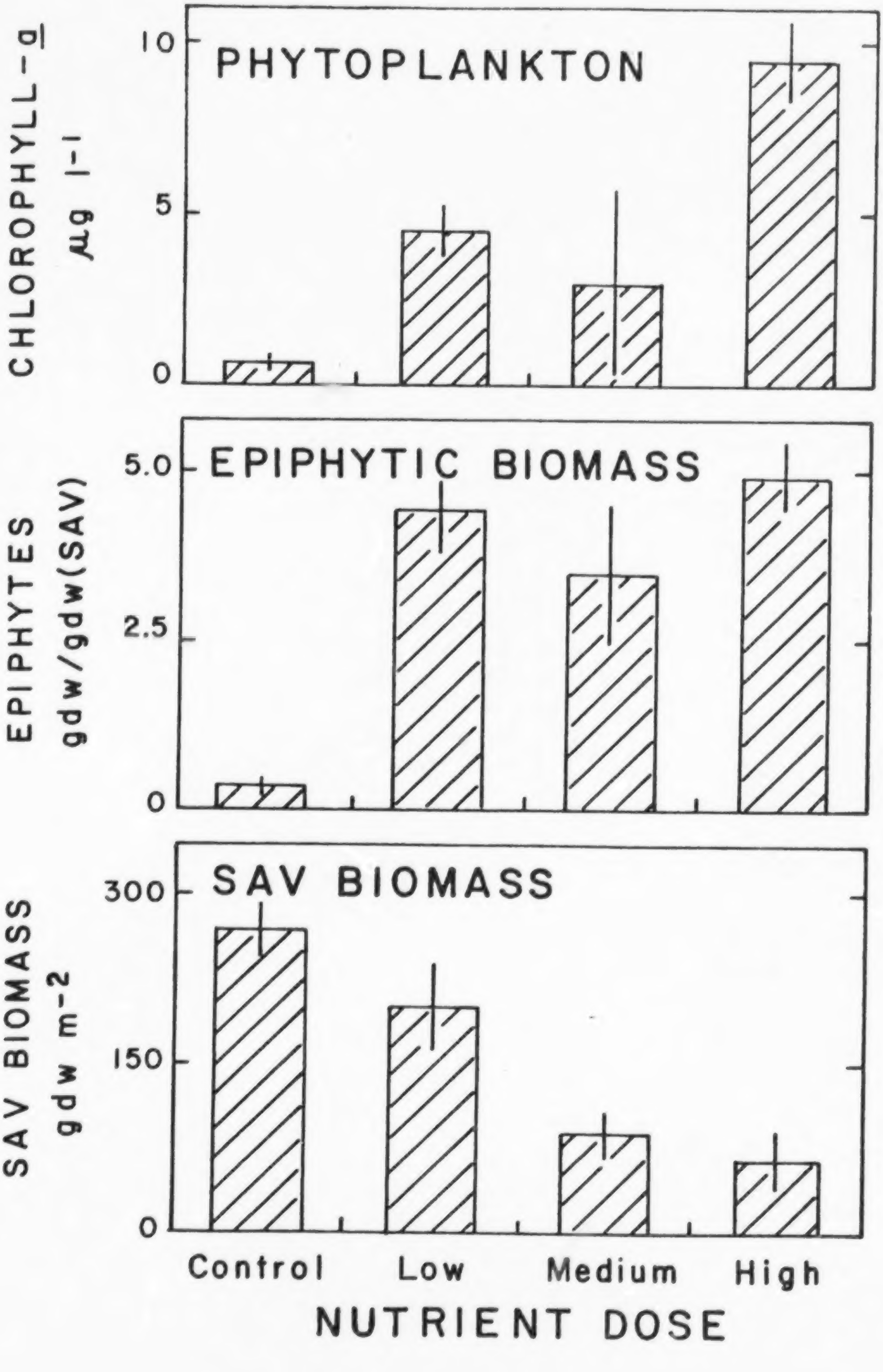
PHYTOPLANKTON
CHLOROPHYLL-a
μg l-1
0
5
10
EPIPHYTIC BIOMASS
EPIPHYTES
gdw/gdw(SAV)
0
2.5
5.0
SAV BIOMASS
SAV BIOMASS
gdw m-2
0
150
300
Control
Low
Medium
High
NUTRIENT DOSE

Fig. 5. Summary of phytoplankton stocks (as chlorophyll a), weight of epiphytic material, and submerged aquatic vegetation biomass in August 1981, for experimental ponds treated with 4 levels of nutrient enrichment after 8 weeks. Given are means 1 standard error (25).

Nutrient Enrichment and SAV Decline

While nutrient fertilization has been occurring in Chesapeake Bay over the last century or more, the upper bay outbreak of M. spicatum in the early 1960's (just preceeding the general SAV decline) may have marked the onset of critical nutrient enrichment levels. It appears that this species has competitive advantage over other SAV under conditions of elevated nutrient inputs (e.g. 52). During the last several years numerous investigators have postulated that eutrophication was responsible for observed losses of SAV in freshwater systems including: Loch Leven, Scotland (38); Lake Erie, Ohio (53); Whitewater Lake, Ontario (54); and Norfolk Broads, England (10). Others have suggested that nutrient enrichment may be one of several factors contributing to dimunition of seagrasses in such coastal ecosystems as the Dutch Waddenzee (9), the French Mediterranean (55) and Western Australia estuaries (56). In all cases, these declines have occurred progressively over decade-long periods, consistent with observations in Chesapeake Bay (13).

In our experimental pond studies, SAV biomass reductions in high dose ponds amounted to a foreshortening of the normal growing sesaon; however, some effects on vegetative reproduction were also apparent. For example, shoot production from budding was markedly reduced in experimental ponds the spring following a summer of high nutrient treatment, and after a second year of identical treatment vegetative root nodules were also a significantly reduced (25). To interpret these results in the context of long-term declines in abundance, it may be necessary to relate premature seasonal scenescence to decreased reproductive success in a more quantitative fashion.

SYNTHESIS OF FINDINGS AND IMPLICATIONS

Ecosystem Modeling as a Tool for Synthesis

The research discussed in this paper has identified and described a wide range of detailed interactions between submerged vascular plants and their environment. We used a variety of numerical simulation models for these SAV ecosystems to place the complex interactions into a unified ecological framework. One of these models was designed to address questions relating to management of SAV resources in the estuary, including both the role of these plants in secondary production and the environmental factors influencing plant growth (57). Generally, this model consisted of 14 simultaneous, non-linear differential equations, with time as the independent variable. Each equation described the temporal behavior of a state variable, with each term in a given equation representing a connection between that variable and another internal or external variable. The model was calibrated with data collected from field and laboratory measurements and was used to interpolate between discrete observations in time and space and to both hindcast and forecast consequences of changing external factors (e.g. temperature, nutrient loading and flushing rate).

Digital computer simulations were performed (using finite difference numerical methods) to consider the effects of herbicide, sediment and nutrient inputs to the estuary individually and combined (Fig. 6). Under simulated conditions based on maximum observed herbicide concentrations in a shallow embayment, annual mean SAV biomass (B) and total net production (NP) decreased by about 2% and 4% respectively (Fig. 6a), with greatest effects during May-June runoff events. A doubling of sediment loading to the SAV bed and surrounding waters resulted in 13% and 22% reductions in B and NP, and the effects were most pronounced in the late summer and fall periods when heavy winds stimualted sediment resuspension (Fig. 6b). A two-fold nutrient enrichment resulted in marked reductions in B and NP (29% and 48%), with strongest depressions occurring in June (Fig. 6c). Apparently, relatively high nutrient and light levels in spring promoted rapid growth of epiphytic algae which was retarded subsequently in summer due to nutrient depletion. These simulation results are similar to findings from pond fertilization experiments (25). Combining these three stresses in a single simulation resulted in an average of 35% and 56% losses of biomass and production, and the net effect of the three factors thus appeared to be somewhat antagonistic (Fig. 6d).

It is interesting to note that under combined stresses the growing season (period of continuous biomass accumulation) was reduced from about 4.5 mo to less than 2 mo. In addition, plant biomass at the end of the simulated year was considerably lower than initial levels under combined factors. While this might suggest an effect on reproductive success of this population, multi-year simulations produced recurring patterns similar to that shown in Fig. 6. However, this is not surprising because reproductive biology, per se, was not an explicit function in the model.

Gradual Reductions in Abundance versus Population Demise

Most of the species occurring in upper Chesapeake Bay undergo clear annual cycles of above-ground structure, and new growth in spring may be generated either from seeds or from underground storage organs. The model simulations indicated that under environmental stress, net growth available for allocation to sexual or vegetative reproduction is reduced. Experimental observations have demonstrated that extreme light stress, related either to turbidity or epiphytic growth, results in significant decreases in both flowering and production of underground storage nodules (25, 32). We hypothesize that the observed gradual reductions in abundance of stressed plants in model and field experiments would eventually result in a local demise of these populations due to reproductive failure. This final outcome would require a repetitive sequence of annual stressed growth conditions, incrementally undermining reproductive vitality.

In general, SAV communities exhibit considerable ability to modify their local environment and thereby reduce stresses; however, this "buffering capacity" can be exceeded and lead to premature seasonal die-back. Two examples involve nutrients and suspended particulates. While SAV communities are capable of rapid uptake of nutrients from overlying water (4), nutrient enrichment beyond the assimilative capacity can promote epiphytic algal growth to the detriment of vascular plant production (e.g. 10, 25). Secondly, the well established ability of seagrasses and other SAV to trap and bind suspended particulate matter (e.g. 5, 58) is limited in silty estuarine environments

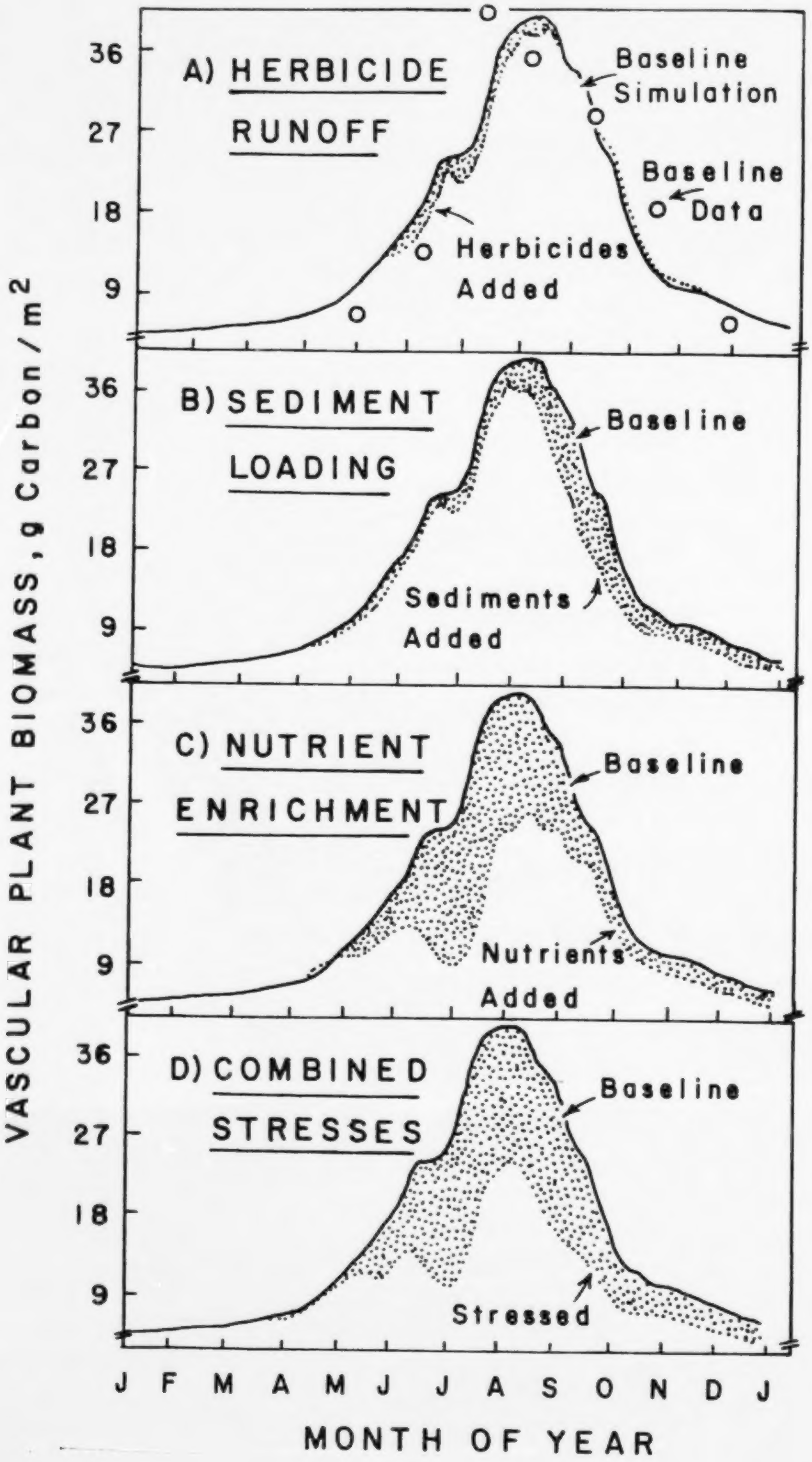
A) HERBICIDE RUNOFF
Baseline Simulation
Baseline Data
Herbicides Added
B) SEDIMENT LOADING
Baseline
Sediments Added
C) NUTRIENT ENRICHMENT
Baseline
Nutrients Added
D) COMBINED STRESSES
Baseline
Stressed
36
27
18
9
VASCULAR PLANT BIOMASS, g Carbon / m²
J F M A M J J A S O N D J
MONTH OF YEAR

Fig. 6. Results of numerical simulations of submerged vascular plant biomass over an annual cycle under conditions of maximum herbicide runoff, doubled sediment loading, doubled nutrient enrichment, and a combination of these three stresses. In all cases the upper line (shown for comparative purposes) represents the baseline conditions which are compared to means of field observations in the upper panel (57).

(25), and associated turbid waters tend to reduce light availability and plant production. It appears that while moderate levels of these stresses result in little if any reductions in plant production and distribution, extreme levels can overwhelm the ability of such plant communities to ameliorate these factors.

Reconstructing the SAV Decline

Historical trends in data for SAV distribution and abundance, sediment and nutrient loading and herbicide use (Fig. 1) coupled with experimental results and model simulations reported here allow development of a plausible, although not conclusive, scenario concerning the observed decline of SAV in Chesapeake Bay.

Accelerated soil erosion and sediment loading associated with extensive land development in the late 1950's apparently led to elevated turbidity in several regions of the estuary (28, 29, 30, 35). Nutrient loading also increased from point sources associated with population centers and from regional diffuse sources associated with intensifying agriculture. In the Patuxent River estuary, marked declines in SAV populations (59) coincided with increased turbidity and nutrient concentrations. In the Potomac River, invasion by M. spicatum followed by proliferation of blue-green algal mats, correlated with expanding sewage discharges and incipient declines in native SAV (60). The establishment of M. spicatum in brackish portions of Chesapeake Bay preceeded a general SAV decline (11), which may have been a response to eutrophication (52). In the late 1960's, it appears that strict erosion control practices reduced sediment runoff inputs and associated turbidities in some regions; however, nutrient inputs continued to rise. The general use of herbicides for agricultural weed control in the watershed in the mid-1960's may have contributed slightly to deteriorate SAV growth conditions, and the greater tolerance of M. spicatum to herbicides is consistent with the observed brief proliferation of this species. Possibly further compounding these stressed conditions was the drought of the 1960's (Fig. 1) which led to increased salinities (61) and probably salinity stress for various freshwater species (62). In the summer of 1972 a 200 year storm event resulted in rates of sediment deposition up to 25 cm (63) in some locations, virtually burying local plant communities. While this storm occurred well after the initial evidence of SAV decline, its effects are apparent in plant abundance data (Fig. 1a). There is also evidence for considerable damage to SAV populations from intense grazing and foraging episodes by waterfowl and fish (64, 65).

While it is impossible to definitely demonstrate what caused the SAV decline, data from controlled experiments, as well as recent field observations and historical records all suggest that nutrient enrichment and increased turbidity probably played major roles. Herbicide runoff may represent an ephemerally and locally important stress, but our data indicate its contribution to this general die-back has been minimal. Major meteorological events and direct grazing certainly have contributed to the rigors of SAV existence and may have accelerated the recent decline, but historical records indicate these factors probably also played a secondary role.

Management of SAV Resources

The potential for improving environmental conditions for propagation and growth of submerged vascular plants in the estuary can be considered in terms of the relative importance and controllability of key factors. Herbicide losses from croplands to adjacent watercourses can be reduced by such measures as alternative weed control techniques and "buffer stripping" at the margins of fields; however, the socio-economic costs of such strategies would be considerable (66). Research results presented here indicate herbicides exert a minor influence on SAV, so that the wisdom of such measures may be questionable.

Three major sources of suspendable sediments to the estuary are runoff, shoreline erosion and resuspension of bottom sediments (67). Both shore erosion, which is important in the middle bay and eastern shore tributaries (67, 68), and resuspension, which dominates in most estuarine shallows (25), are largely uncontrollable. Existing soil conservation practices have been reasonably effective in reducing sediment runoff (29, 30) and probably represents a realistic management option, given the apparent contribution of suspendable sediments to the SAV decline.

Although nutrient inputs to Chesapeake Bay from sewage effluents represent less than 20% of the total for nitrogen (69), in some areas such as the Potomac and Patuxent Rivers, they are a major source which can be controlled, albeit at considerable cost. Diffuse sources, dominated by agricultural runoff, represent the largest nutrient input to the estuary, and efforts to develop effective controls are crucial. The substantial experimental and correlative data available strongly suggest that nutrient enrichment has resulted in losses of submerged vascular plants in a variety of aquatic systems, including Chesapeake Bay. This, coupled with other documented consequences of excessive fertilization (e.g. noxious algal blooms and anoxic bottom waters) point to a considerable need to focus on nutrient waste management.

The role of SAV communities in maintaining water quality and promoting secondary production has been well established in general and in Chesapeake Bay in particular. For example, it has been estimated that about 50% of the current sewage derived nutrient inputs to the bay could have been assimilated by healthy SAV communities which existed in 1960 (70). Kahn (71) has calculated that previous SAV populations supported a fishing resource valued in excess of a million USA dollars per year. Nevertheless, estuaries are characteristically stressed environments, and such natural stresses combined with anthropogenic factors tend to make estuarine littoral regions only marginally compatible with SAV physiological requirements. If a decision were made to attempt a reversal of this SAV decline, it appears that several steps would be required. First, nutrient inputs should be reduced and efforts should be made to continue and improve soil erosion control practices. Second, a better understanding of propagation and reproduction mechanisms and capabilities of these plants is needed. Finally, a substantial program (possibly involving direct transplanting) might be required to accelerate restoration of these SAV communities.

REFERENCES

1. Hutchinson, G.E. 1975. A treatise in limnology. Vol. 3. Limnological botany. John Wiley, New York.

2. Thayer, G.W., D.A. Wolfe, and R.B. Williams. 1975. The impact of man on seagrass systems. Ameri. Scientist 63:288-96.

3. Den Hartog, C. 1977. Structure, function and classification in seagrass communities, pp. 89-121 In: C.P. McRoy and C. Helfferich (eds.) Seagrass ecosystems: A Scientific perspective. Marcel Dekker, New York.

4. Howard-Williams, C. 1981. Studies on the ability of a Potamogeton pectinatus community to remove dissolved nitrogen and phosphorus compounds from lake water. J. Appl. Ecol. 18:619-637.

5. Orth, R.J. 1977. The importance of sediment stability in seagrass communities, pp. 281-300 In: B.C. Coull (ed) Ecology of marine benthos. Univ. S. Carolina Press, Columbia.

6. Ott, J.A. 1980. Growth and production in Posidonia oceanica. Marine Ecol. (P.S.Z.N.I.) 1:47-64.

7. Milne, L.J. and M.J. Milne. 1951. The eelgrass catastrophe. Sci. Amer. 184:52-55.

8. Rasmussen, E. 1977. The wasting disease of eelgrass (Zostera marina) and its effects on environmental factors and fauna, pp. 1-52 In: C.P. McRoy and C. Helfferich (eds.) Seagrass ecosystems: A scientific perspective. Marcel Dekker, New York.

9. Den Hartog, D. and P.J.G. Polderman. 1975. Changes in the seagrass populations of the Dutch Waddenzee. Aquat. Bot. 1:141-147.

10. Phillips, G.L., D. Eminson and G. Moss. 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. 4:103-126.

11. Bayley, S., V.D. Stotts, P.F. Springer, J. Steenis. 1978. Changes in submerged aquatic macrophyte populations at the head of the Chesapeake Bay, 1958-1975. Estuaries 1:74-85.

12. Stevenson, J.C. and N.M. Confer. 1978. Summary of available information on Chesapeake Bay submerged vegetation. U.S. Dept. Inter. FWS/OBS-78/66. NTIS, Springfield, VA. 333 pp.

13. Orth, R.J. and K.A. Moore. 1981. Submerged aquatic vegetation of the Chesapeake Bay: Past, present and future. Trans. N. Amer. Wildl. Nat. Res. 46:271-283.

14. Brush, G.S., F.W. Davis and C.A. Stenger. 1981. Sediment accumulation and the history of submerged aquatic vegetation in the Patuxent and Ware Rivers: A stratigraphic study. US EPA Final Rep., Grant No. R80668001, Annapolis, MD.

15. Boynton, W.R., W.M. Kemp and J.C. Stevenson. 1979. A research design for understanding and managing complex environmental resource systems, pp. 169-177 In: R.R. Johnson and J.F. McCormick (eds.) Strategies for protection and management of flood plain wetlands and other riparion ecosystems. (GTR-WO-12). U.S. Dept. Agric., Forest Serv., Washington, DC.

16. Kemp, W.M., M.L. Lewis, J.J. Cunningham, J.C. Stevenson, W.R. Boynton. 1980. Microcosms, macrophytes, and hierarchies: Environmental research in Chesapeake Bay, pp. 911-936 In: J. Giesy (ed) Microcosm research in ecology. ERDA Conf. 781101. NTIS, Springfield, VA.

17. Kemp, W.M., J.C. Means, T.W. Jones and J.C. Stevenson. 1982. Herbicides in Chesapeake Bay and their effects on submerged aquatic vegetation, pp. 503-567. In: U.S. Environ. Protect. Agen. Chesapeake Bay Program technical studies: A synthesis. U.S. Govt. Printing Office, No. 509-660. Washington, DC.

18. Newby, L.C., R.A. Kahrs, K. Adams and M. Szolics. 1978. Atrazine residues in the Chesapeake Bay. Proc. N.E. Weed Sci. Soc. 32:339 (Abstract).

19. Zahnow, E.W. and J.C. Riggleman. 1980. Search for linuron residues in tributaries of the Chesapeake Bay. J. Agric. Food Chem. 28:974-978.

20. Means, J.C., R. Wijayaratne and W.R. Boynton. 1983. Fate and transport of selected pesticides in estuarine environments: Implications for ocean pollution. Can. J. Fish. Aquat. Sci. (In press).

21. Wu, T.L. 1980. Dissipation of the herbicides atrazine and alachlor in a Maryland corn field. J. Environ. Qual. 9:459-465.

22. Hershner, C., K. Ward and J. Illowsky. 1981. The effects of atrazine on *Zostera marina* in the Chesapeake Bay, Virginia. Rept. to US EPA. Virginia Instit. Marine Sci., Gloucester Pt., VA.

23. Jones, T.W., W.M. Kemp, J.C. Stevenson and J.C. Means. 1982. Degradation of atrazine in estuarine water/sediment systems and selected soils. J. Environ. Qual. 11:632-638.

24. Means, J.C. and R. Wijayaratne. 1982. Role of natural colloids in transport of hydrophobic pollutants. Science 215:968-970.

25. Kemp, W.M., W.R. Boynton, R.R. Twilley, J.C. Stevenson and J.C. Means (eds.). 1982. Submerged aquatic vegetation in upper Chesapeake Bay. Final Report Univ. Md. Center Environ. Estuar. Stud. Ref. No. HPEL 82-193, Cambridge, MD.

26. Forney, D.R. and D. Davis. 1981. Effects of low concentrations of herbicides on submersed aquatic plants. Weed Sci. 29:677-85.

27. Correll, D.L. and T.L. Wu. 1982. Atrazine toxicity to submersed vascular plants in simulated estuarine microcosms. Aquatic Bot. 14:151-158.

28. Wolman, G.W. and A.P. Shick. 1967. Effects of construction and fluvial sediment form urban and suburban areas of Maryalnd. Water Resources Res. 3:451-464.

29. Roberts, W.P. and J.W. Pierce. 1974. Sediment yield in the Patuxent River (MD) undergoing urbanization, 1968-1969. Sedimentary Geol. 12:179-197.

30. Roberts, W.P. and J.W. Pierce. 1976. Deposition in upper Patuxent Estuary, Maryland, 1968-1969. Est. Coast. Mar. Sci. 4:267-280.

31. Kemp, W.M., W.R. Boynton, J.C. Stevenson and J.C. Means (eds.). 1981. Submerged aquatic vegetation in Chesapeake Bay. Univ. Md. Center Environ. Estuar. Stud., Ref. No. HPEL-81-28, Cambridge, MD.

32. Goldsborough, W.J. 1983. Light and shade adaptation in the submerged vascular plant, Potamogeton perfoliatus. MS Thesis, Univ. MD, College Park.

33. Mihursky, J.A. and W.R. Boynton. 1978. Review of Patuxent Estuary data base. Univ. MD Center Environ. Estuar. Stud. Ref. No. 78-157-CBL, Solomons, MD.

34. Lorenzen, C.J. 1972. Extinction of light in the ocean by phytoplankton. J. Cons. Int. Expor. Mer. 34:262-267.

35. Heinle, D.R., C.F. D'Elia, J.L. Taft, J.S. Wilson, M. Cole-Jones, A.B. Caplins and L.E. Cronin. 1980. Historical review of water quality and climatic data from Chesapeake Bay with emphasis on effects of enrichment. Univ. MD Center Environ. Estuar. Stud. Ref. No. 80-15-CBL, Solomons, MD.

36. Carignan, R. and J. Kalff. 1979. Quantification of sediment phosphorus available to aquatic macrophytes. J. Fish. Res. Bd. Can. 36:1002-1005.

37. Iizumi, H. and A. Hattori. 1981. Growth and organic production of eelgrass (Zostea marina) in temperate waters of the Pacific Coast of Japan. III. The kinetics of nitrogen uptake. Aquat. Bot. 12:245-256.

38. Jupp, B.P. and D.H.N. Spence. 1977. Limitations on macrophytes in a eutrophic lake, Loch Leven. J. Ecol. 65:175-186.

39. Sand-Jensen, K. 1977. Effect of epiphytes on eelgrass photosynthesis. Aquat. Bot. 3:55-63.

40. Penhale, P. 1977. Macrophyte-epiphyte biomass and productivity in an eelgrass (Zostera marina) community. J. Exp. Mar. Biol. Ecol. 26:211-224.

41. McNulty, J.K. 1961. Ecological effects of sewage pollution in Biscayne Bay, Florida. Bull. Mar. Sci. Gulf Carib. 11:394-447.

42. Correll, D.L. 1981. Nutrient mass balances for the watershed, headwaters, intertidal zone, and basin of the Rhode River Estuary. Limnol. Oceanogr. 26:1142-1149.

43. O'Brien, W.J. and F. deNoyelles. 1974. Relationship between nutrient concentration, phytoplankton density, and zooplankton density in nutrient enriched experimental ponds. Hydrobiologia 44:105-125.

44. Edmondson, W.T. and Y.H. Edmondson. 1947. Measurements of production in fertilized salt water. J. Mar. Res. 6:228-245.

45. Menzie, C.A. 1979. Growth of the aquatic plant Myriophyllum spicatum in a littoral area of the Hudson River estuary. Aquat. Bot. 6:365-375.

46. Moss, B. 1976. The effects of fertilization and fish on community structure and biomass of aquatic macrophytes and epiphytic algal populations: An ecosystem experiment. J. Ecol. 64:313-342.

47. Mulligan, H.F., A. Baronowski and R. Johnson. 1976. Nitrogen and phosphorus fertilization of aquatic vascular plants and algae in replicate ponds. I. Initial response to fertilization. Hydrobiologia 48:109-116.

48. Scott, B.C. 1978. Phytoplankton distribution and light attenuation in Port Hacking estuary. Aust. J. Mar. Freshwater Res. 29:31-44.

49. Bannister, T.T. 1974. A general theory of steady state phytoplankton growth in a nutrient saturated mixed layer. Limnol. Oceanogr. 19:13-30.

50. Borum, J. and S. Wium-Anderson. 1980. Biomass and production of epiphytes on eelgrass (Zostera marina) in the Øresund, Denmark. Ophelia (Suppl.) 1:57-64.

51. Sand-Jensen, K. and M. Søndergaard. 1981. Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. Int. Rev. ges. Hydrobiol. 66:529-552.

52. Lind, C.T. and G. Cottam. 1969. The submerged aquatics of University Bay: A study in eutrophication. Amer. Midl. Nat. 81:353-369.

53. Stuckey, R.L. 1978. The decline of lake plants. Natural History 87:66-69.

54. Dale, H.M. and G.E. Miller. 1978. Changes in the aquatic macrophyte flora of Whitewater Lake near Sudbury, Ontario from 1947 to 1977. Canad. Field-Naturalist 92:264-270.

55. Perés, J.M. and J. Picard. 1975. Causes de la rarefaction et de la disparition des herbiers de *Posidonia oceanica* sur les cotes Francaises de la Mediterranee. Aquatic Bot. 1:133-139.

56. Cambridge, M.L. 1979. Cockburn Sound study technical report on seagrass. Dept. Conservation and Environment, Western Australia. Rep. No. 7.

57. Kemp, W.M., A.J. Hermann and W.R. Boynton. 1981. Resource dynamics and ecology of submerged aquatic vegetation in Chesapeake Bay: A modeling approach to demonstrate resource management concepts. Univ. MD Center Environ. Estuar. Stud. Ref. No. HPEL-81-214, Cambridge, MD.

58. Ginsburg, R.N. and H.A. Lowenstam. 1958. The influence of marine bottom communities on the depositional environment of sediments. J. Geol. 66: 310-318.

59. Anderson, R.R. 1966. Plant ecology of the upper Patuxent River Estuary with special reference to the effects of thermal pollution on macrophytes. Ph.D. Thesis, Univ. MD, College Park, 99 p.

60. Jaworski, N.A., D.W. Lear and O. Villa. 1972. Nutrient management in the Potomac estuary, pp. 246-273 *In*: G.E. Likens (ed) Nutrients and eutrophication. Am. Soc. Limn. Oceanog. Publ., Allen Press, Lawrence, KA.

61. Ritchie, D.E. and J.B. Genys. 1975. Daily temperature and salinity of surface waters of Patuxent River at Solomons, MD, based on 39 years of records. Ches. Sci. 16:127-133.

62. Haller, W.T., D.L. Sutton and W.C. Barlowe. 1974. Effects of salinity on growth of several aquatic macrophytes. Ecology 55:891-894.

63. Schubel, J.R. and D.J. Hirschberg. 1978. Estuarine graveyards, climatic change and the importance of the estuarine environment, pp. 285-303. *In*: M.L. Wiley (ed) Estuarine interactions. Academic Press, New York.

64. Kiørboe, T. 1980. Distribution and production of submerged macrophytes in Tippen Grund, and the impact of waterfowl grazing. J. Appl. Ecol. 17:675-687.

65. Orth, R.J. 1975. Destruction of eelgrass, *Zostera marine*, by the cownose ray, *Rhinoptera bonasus*, in the Chesapeake Bay. Ches. Sci. 16:205-208.

66. Stewart, B.A. 1975. Control of water pollution from croplands, Vol. 1. USDA Rept. ARS-H-5-1, US EPA Rept. EPA-600/2-75-026a. NTIS, Springfield, VA, 111 p.

67. Biggs, R.B. 1970. Sources and distribution of suspended sediment in northern Chesapeake Bay. Mar. Geol. 9:187-201.

68. Yarbro, L.A., P.R. Carlson, R. Crump, J. Chanton, T.R. Fisher, N. Burger and W.M. Kemp. 1981. Seston dynamics and a seston budget for the Choptank River Estuary in Maryland. Univ. Md. Center Environ. Estuar. Stud. Ref. No. HPEL-81, Cambridge, MD.

69. Smullen, J.T., J.L. Taft and J. Macknis. 1982. Nutrient and sediment loads to the tidal Chesapeake Bay system, pp. 147-262. In: US Environ. Protect. Agen. Chesapeake Bay Program technical studies: A synthesis. US Govt. Printing Office No. 509-660. Washington, DC.

70. Boynton, W.R. and K.L. Heck. 1982. Ecological role and value of submerged macrophyte communiteis, pp. 428-502. In: US Environ. Protect. Agen. Chesapeake Bay Program technical studies: A synthesis. US Govt. Printing No. 509-660. Washington, DC.

71. Kahn, J.R. 1981. The economic losses associated with depletion of submerged aquatic vegetation in Chesapeake Bay. Ph.D. Thesis. Univ. MD, College Park, 120 p.